Evo-Devo: the Merging of Evolutionary and Developmental Biology

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Introduction

In the beginning of the 20th century, developmental biology was at the forefront of biology, but then declined and had a renaissance towards its end. The key to this revival were the techniques of molecular biology, which proved the great equalizer for all branches of biology. The fusion of molecular, developmental and evolutionary biology proved very fertile, and led to the birth of a new discipline, Evo-Devo. I would like to present a personal account on how this synthesis took place.

We will consider here three main points:

1) How are the mechanisms of self-regulation of cell differentiation observed in animal development explained at the molecular level?
2) How were conserved ancestral gene networks common to all animals – which pattern the Antero-Posterior (A-P) and Dorso-Ventral (D-V) axes – used to generate the immense variety of animal forms?
3) How has the use of a common tool-kit of genes present in the ancestral animal genome channeled the outcomes of evolution through natural selection?

The main conclusion that emerges from these genomic, developmental and evolutionary studies is that all bilateral animals – which comprise 30 of the 34 extant phyla – arose through gene mutation, duplication or deletion of the genome of a complex common ancestor, the *Urbilateria* (Ur: primeval; Bilateria: animals having bilateral symmetry).

1. Self-regulation of differentiating cell fields

1.1. Embryology at the forefront of biology

When biologists realized that it was necessary to take an experimental – rather than descriptive – approach to understand the mechanisms of development, embryology rapidly became the leading edge of biological studies. Embryos offer excellent material for experimental biology.

After fertilization, an amphibian egg – a large cell 1.2 mm or more in diameter – divides synchronously into 2, 4, 8, 16, 32, 64 and so on cells. At

these early stages, cells are dedicated to sensing their position within the embryo by signaling to each other without differentiating into particular tissues. At the 10,000 cell stage, cells on the dorsal side start to invaginate to the interior of what at this point constitutes a blastula or hollow ball. The cells that involute will form the endoderm and mesoderm of the body, while cells that remain on the outside give rise to ectoderm. By the end of this process – called gastrulation – a vertebrate embryo with defined A-P and D-V axes and differentiated tissue types is formed.

The beginning of experimental embryology can be traced back to 1891, when Hans Driesch separated the first two cells of a sea urchin embryo and obtained two complete larvae. At the turn of the century, in 1901, Hans Spemann obtained amphibian twins by gently constricting embryos with fine ligatures of hair from his newborn daughter. Much later, I found that identical twins can also be generated by simply bisecting an early embryo of the frog *Xenopus laevis* with a scalpel blade before gastrulation starts.

This tendency of the embryo to regenerate towards the whole is called self-regulation. This is not a property restricted to the early embryo. Most organs in the body start their development as 'morphogenetic fields' that are able to self-regulate their differentiation. This was discovered by Ross G. Harrison, who showed in 1918 that a circular region of flank mesoderm could induce the development of forelimbs when transplanted into a host embryo. When he cut this region in half, each half induced a limb. Not a half-limb, but rather a complete limb. From these transplantation experiments we learned that cells within the organism do not lead solitary lives, but are instead subsumed in larger fields of hundreds or thousands of cells that communicate to each other when to proliferate, differentiate, or die. We are only now beginning to understand the molecular mechanisms by which these cellular conversations take place.

1.2. Hans Spemann and embryonic induction

The way forward in the analysis of self-regulation of pattern came from a transplantation experiment carried out by a graduate student at Freiburg University, Hilde Mangold. Under the direction of Spemann, she transplanted the dorsal lip of the blastopore, the region in which the involution of mesoderm starts, and introduced it into the opposite (ventral) side of a host embryo. With a gentle push, the embryonic fragments heal together almost miraculously, and two days later perfect Siamese (conjoined) twins are formed. Spemann called this dorsal region of the embryo the 'organizer'.

Remarkably, the transplanted organizer cells themselves gave rise to notochord, yet were able to induce their neighboring cells to change their

differentiation into dorsal tissues such as central nervous system (CNS), somite (muscle), and kidney. Therefore, within the embryo, groups of cells (called organizing centers) are able to instruct their neighbors on the type of cell differentiations they should adopt.

Spemann was awarded the 1935 Nobel Prize for Physiology or Medicine for the discovery of embryonic induction by organizer tissue, which marked the apogee of experimental embryology. However, the isolation of the chemical substances responsible for embryonic induction proved impossible given the methods available at the time. After that, the genetics of Thomas Hunt Morgan became the pre-eminent biological discipline for most of the 20th century.

2. The ancestral A-P and D-V gene networks

2.1. Thomas Morgan, Edward Lewis and homeotic mutations

Morgan started his career as an embryologist. For example, he demonstrated that a 2-cell frog egg could self-regulate to form a whole embryo after killing one cell, but only when the dead cell was removed. He realized, however, that mechanistic progress using this type of experimental approach would be very difficult, and decided to study mutations in the fruit fly *Drosophila melanogaster* instead. Together with his graduate student Calvin Bridges, in 1923 Morgan isolated a mutant, *bithorax*, which gave rise to four-winged flies (flies normally have only two wings). This mutant was to provide the key that made possible the molecular analysis of development.

In 1946, a young student at Caltech, Edward B. Lewis, initiated studies on the genetics of the *bithorax* locus, which continued until his passing in 2004. He found that the *bithorax* region patterned the thorax and abdomen of the fruit fly and contained several genes. When mutated, these genes caused homeotic transformations, i.e., the transformation of one region of the body into the likeness of another region. For example, the third thoracic segment may become transformed into the second thoracic, thus generating the four-winged flies.

Remarkably, Lewis noted that the arrangement of homeotic genes in the DNA followed the same order in which they regulated the A-P identity of abdominal segments. He designated this surprising organization colinearity. Lewis proposed that homeotic genes had repressed thoracic identity in a centipede-like ancestor, and that recent duplications of these genes had further elaborated the identity of each abdominal segment.

When molecular biology became practical, the race to clone a homeotic gene began in several laboratories. It culminated with the isolation of *Anten-*

napedia, a homeotic gene that can transform antenna into leg, independently by Scott and Kaufman, and by Garber and Gehring in 1983. Searching for the hypothetical recently duplicated genes of Lewis, they discovered that many *Drosophila* homeotic genes crossreacted with a short region of DNA. This conserved segment of nucleic acid, called the homeobox, was found to encode a DNA-binding domain of 60 amino acids, designated the homeodomain.

2.2. Hox genes in vertebrates

At that time I was a professor in the same department as Walter Gehring at the Biozentrum of the University of Basel, Switzerland, and we shared group meetings. We decided to collaborate to test whether homeobox genes might be present in vertebrates. (The experiment was conceived for the wrong reasons: the first expression studies by Garber had shown *Antennapedia* expression in the CNS, and we suspected it might encode a peptide hormone, which were known at the time to have been conserved between Hydra and mammals). On the first try we cloned a gene, now called HoxC-6, from a *Xenopus laevis* genomic library which crossreacted with *Antennapedia* and *ultrabithorax* (Carrasco *et al.*, 1984). The sequence of the homeodomain was very similar to that of *Antennapedia*. Later gene knockout studies by Mario Capecchi and others showed that this gene, like the other 39 Hox genes, caused A-P homeotic transformations when mutated in the mouse. This was a good thing, because in our paper in the last sentence of the introduction I had written: 'If the frog gene cloned here eventually turns out to have functions similar to those of the fruit fly genes, it would represent the first development-controlling gene identified in vertebrates'. And so it was.

Vertebrate Hox genes are clustered in the genome. Work by other groups, mostly in mouse embryos, showed that vertebrate Hox gene expression in the body is colinear with their order in the DNA. The homeobox sequences and overall organization of the vertebrate Hox gene complexes were conserved with those of *Drosophila* and other invertebrates. Therefore, Lewis' hypothesis that homeotic genes were recently duplicated genes was not correct, yet provided the cornerstone for the new discipline of Evo-Devo. Edward Lewis received the Nobel Prize for Medicine or Physiology for his work on developmental genetics in 1995.

2.3. Whole-genome duplications in the vertebrate lineage

Many insects have eight or so Hox genes arranged in a single cluster. *Amphioxus*, a chordate closely related to the vertebrates, has a single cluster containing 14 Hox genes in a row. However, the situation is more complex in the vertebrates. This is because vertebrates underwent two rounds of

whole-genome duplications at the beginning of their evolution. Thus, for each gene humans may have up to four copies. Many of our genes are now present as single copies, but this only indicates that the other three were lost. Gene loss is easily achieved over evolutionary time. Duplicated genes are retained when a duplicated copy acquires a specialized function that makes it beneficial for the survival of the species. These two genome-wide duplications were probably a crucial event in the remarkable evolutionary success of vertebrate animals.

Humans contain four Hox gene complexes, called HoxA through HoxD. Each consists of about 100,000 base pairs of DNA and resulted from the duplication of an ancestral Hox complex containing 13 genes. However, instead of $13 \times 4 = 52$, humans retained a total of only 39 Hox genes. This is because some Hox genes were deleted. As will be discussed below, gene loss is an important force in shaping evolution.

The degree of conservation between these four mammalian Hox complexes and *Drosophila* is simply amazing. Not only homeobox sequences and colinearity of expression patterns were maintained, but even their regulation by an inhibitory microRNA (called *infra-abdominal-4* in *Drosophila* and miR196 in humans) was conserved.

This intricate genetic machinery that patterns the A-P axis could not have been assembled independently twice in *Drosophila* and vertebrates, let alone in all phyla. The only reasonable interpretation is that a Hox complex was already functional in *Urbilateria* and was inherited by its descendants. The discovery of conserved Hox gene complexes led to the realization that the gene networks that control the A-P axis share deep historical homologies. Before the discovery of the homeobox we did not imagine that the mechanisms of development would be so similar between fruit flies and humans. It was a great surprise.

2.4. François Jacob's symposium on Evolution and Development

In 1991, a landmark meeting was held in Crete. Organized, among others, by academicians Nicole Le Douarin and Fotis Kafatos, it was entitled *Evolution and Development*. Its topic had been specifically requested by François Jacob, who was retiring. Jacob, a great geneticist, was very interested in evolution. In his excellent book, *The Possible and the Actual* (1982), Jacob explained why bringing these two separate fields together was important: 'For it is during embryonic development that the instructions contained in the genetic program of an organism are expressed, that the genotype is converted into phenotype. It is mainly the requirements of embryonic development that, among all posible changes in genotype, screen the actual

phenotypes'. The main argument of his book was that during evolution old components are retained and used again, comparing evolution to the work of a tinkerer or *bricoleur*. A tinkerer uses parts or materials that already exist to assemble objects having new purposes.

Jacob displayed great insight in bringing together developmental and evolutionary biologists as his swan's song. The symposium took place at the perfect time, when the conservation of the Hox system was already understood in general outlines. The star of the meeting was paleontologist Stephen Jay Gould. Wishing to learn more about evolution, I asked him to sit at my table during breakfast. Although he really wanted to read his newspaper in peace, I proved too eager and he reluctantly accepted. Gould recommended I should read two books. The first one was Gould's own *Wonderful Life*, which told the story of the Cambrian explosion in the fossil record.

The Cambrian explosion refers to the remarkable finding that all the body plans (34 phyla) of animals that exist today appeared as fossils over a narrow period of time, between 535 to 525 million years ago. Before that time a long line of Precambrian ancestors must have existed, but they left very few or no adult bilaterian fossils (except for tracks and trails in the ocean floor dating to 630 million years ago). We do not know why the appearance of body plans occurred so suddenly, and many possibilities have been proposed (Valentine, 2004). For example, in the 'snowball earth' scenario the diversification of body plans resulted from repeated bottlenecks of intense natural selection coinciding with several massive glaciation events that covered most of the earth between 750 and 550 million years ago. Even more mysterious than the sudden emergence of phyla, is the question of why no new animal body plans have evolved since then, for which we currently have no answers.

2.5. Geoffroy Saint-Hilaire and the unity of plan

The second book that Gould recommended was one by Toby Appel, on the historical debate that took place at the French Academy of Sciences between Georges Cuvier and Etienne Geoffroy Saint-Hilaire in 1830. Geoffroy held the view that a unity of plan existed among animals. In 1822, he dissected a lobster and placed it in an inverted position with respect to the ground. In this upside down orientation the lobster's normally ventral nerve cord was located above the digestive tract, which in turn was placed above the heart. In his own words: 'What was my surprise, and I add, my admiration, in perceiving an ordering that placed under my eyes all the organic systems of this lobster in the order in which they are arranged in mammals?'

Geoffroy went on to argue that there was a unity of plan, or design, among animals, so that the dorsal side of the vertebrates was homologous to the ven-

tral side of the arthropods. For historians of science the Cuvier-Geoffroy debate was of great interest because it took place decades before Charles Darwin published his *Origin of Species* in 1859. For our own work, reading this book was crucial, because when a few years later we isolated Chordin, we were prepared. Chordin was a dorsal protein secreted by Spemann's organizer that had a close homologue in the ventral side of the *Drosophila* early embryo.

At Jacob's symposium I presented the first investigations from our laboratory on the chemical nature of embryonic induction by Spemann's organizer. At that time, we had constructed libraries containing the genes expressed in dorsal lips manually dissected from the frog gastrula. We had just isolated a gene expressed exclusively in organizer tissue called *goosecoid*. It encoded a DNA-binding protein, but we knew from Spemann's work that embryonic induction required secreted factors able to change the differentiation of neighboring cells.

By continuing these explorations on the molecular nature of induction by organizer tissue, we isolated several secreted proteins such as Chordin, Frzb-1 and Cerberus, and other groups isolated Noggin, Follistatin and Dickkopf (De Robertis, 2006). Unexpectedly, all of these proteins turned out to function as antagonists of growth factors in the extracellular space. They prevent binding of growth factors to their receptors on the cell membrane, thus inhibiting signaling. Although we had hoped to isolate novel signaling growth factors from the organizer, what was discovered instead was that embryonic induction was mediated mainly through the secretion of a cocktail of inhibitory proteins.

2.6. Chordin, BMP and cell differentiation

Chordin proved to be the most informative of the organizer factors. Transplanted organizers in which Chordin expression is inhibited lost all embryonic induction activity. Thus, Chordin is essential for organizer function. Chordin induces the differentiation of dorsal tissues (such as CNS or muscle) by binding to Bone Morphogenetic Proteins (BMPs), which normally cause the differentiation of ventral tissues (such as epidermis or blood). Two BMP genes are expressed in the ventral region of the embryo, and Chordin is secreted in prodigious amounts by dorsal cells. In principle, this would suffice to establish a gradient of BMP activity, yet by further investigating the system we discovered much more complexity.

Dorsal-ventral tissue differentiation results from a biochemical network of proteins secreted by the dorsal and ventral sides of the embryo. For each action of the dorsal organizer there is a compensating reaction in the opposite side of the embryo. The expression of genes on the dorsal and the ventral sides are

under opposite control, which explains in part the self-regulation phenomenon. The dorsal side also expresses BMPs, which when bound to Chordin are able to flow towards the ventral side. There, a protease called Tolloid specifically degrades Chordin, liberating BMPs for signaling through its cell surface receptors. The flow of Chordin and its cleavage by this protease are key steps in maintaining the self-regulating gradient of BMP activity. A number of additional secreted proteins (called Sizzled, Crossveinless-2, Twisted gastrulation and Crescent) function as feedback regulators, providing additional resilience to the D-V patterning system (De Robertis, 2009).

Remarkably, other investigators found that this basic biochemical network is also used to regulate cell differentiation along the D-V axis in the early embryos of many other organisms, such as *Drosophila*, beetles, spiders, hemichordates, amphioxus, zebrafish and chick. This intricate molecular machinery is most unlikely to have evolved independently multiple times during evolution specifically to control D-V patterning. The reasonable conclusion is that the Chordin/BMP/Tolloid pathway patterned the dorsal-ventral axis of the last common bilaterian ancestor and was inherited by its descendants.

The conservation of the Chordin/BMP/Tolloid system provided strong molecular support for the hypothesis of Geoffroy Saint-Hilaire that the mammalian and arthropod body plans are homologous. An inversion of the D-V axis occurred during evolution. The ventral side of the arthropods is equivalent to the dorsal side of the vertebrate, and the entire Chordin/BMP/Tolloid pathway was inverted. In both vertebrates and invertebrates, the CNS is formed where the gradient of BMP signaling is lowest. A unity of plan, both for the A-P and D-V axes, exists among animals.

3. A conserved gene tool-kit generates variety in evolution

3.1. Urbilateria had considerable regulatory complexity

These deep homologies in the way all embryos pattern their A-P and D-V axes are having a profound impact on current evolutionary thinking. One might argue that the power of natural selection of the fittest, working on chance mutations over immense periods of geological time, is *per se* sufficient to explain the variety of animal forms. In the absence of any constraints, competition in crowded ecosystems, particularly among closely related species, would lead to new and improved animal designs in the victorious species, through the creative force of natural selection. Ever more adapted generations would be formed because the invisible guiding hand

of natural selection integrates useful mutational changes, forming ever fitter individuals and gradually generating new structures and species. On the other hand, what we are now learning is that a very important source of variation for specifying the arrangements of cells with respect to each other – which is what ultimately determines morphological change – resides in the ancestral developmental gene networks shared by all animals.

3.2. Eyes have a common origin

One might argue that while the Hox and Chordin/BMP gene networks are complex, they could have been used to pattern a very simple ancestral animal. However, there are reasons to think that *Urbilateria* was anatomically complex. One such reason is provided by the ancestral eye structures.

An important problem in evolution is whether adaptations arise through homology or convergence. Homology means that two structures are derived from an ancestral one present in a common ancestor. An example of homology could be the hoof of a horse and the middle digit of the ancestors from which it evolved. Convergence occurs when similar solutions are reached to resolve common functional needs. An example could be the wings of various animal groups, which evolved at very different times but represent similar solutions to a functional requirement. Distinguishing between homology and convergence in evolution can be very difficult. Now molecular biology gives us a historical record of how evolution took place. In the case of animal eyes, conventional wisdom was that animal eyes had arisen independently 40 to 60 times through convergent evolution to fulfill the need for vision.

In 1994 Walter Gehring's group isolated the *eyeless* gene from *Drosophila* and found it had homology to the mammalian *Pax6* homeobox gene. In the mouse, mutations in *Pax6* caused the *small eye* phenotype. In humans, the *Aniridia* gene corresponded to *Pax6*. When mouse *Pax6* was artificially expressed in the antenna or leg precursors of *Drosophila* embryos, it caused the formation of ectopic eyes (Gehring, 1998). Of course, these were *Drosophila* eyes, not mouse ones. In the reciprocal experiment, overexpression of *Drosophila eyeless/Pax6* induced eyes in microinjected frog embryos. The eyes of the clam *Pecten,* and even those of jellyfish, also express *Pax6*. The reasonable conclusion is that all eyes are derived from an ancestral eye that expressed *Pax6*.

3.3. The urbilaterian CNS was anatomically elaborate

One might argue that the eye of *Urbilateria* could have been a very simple photoreceptor cell. However, this does not seem to be the case. We now have a very detailed understanding of the molecular switches (called transcription factors) that control the differentiation of the different neurons of the retina,

which derives from the forebrain. The morphology of mammalian and *Musca domestica* eyes had been described in loving detail by Santiago Ramón y Cajal. In 1915, he noted that by simply displacing the cell body (soma) of two neurons in *Musca*, leaving the cell projections and synaptic connections in place, the entire arrangements of intricate neural connections was maintained, with only small variations, between flies and humans. Recent studies have shown that the transcription factors expressed by various mammalian retinal neurons (photoreceptors, bipolar, and retinal ganglion cells) are replaced in the predicted corresponding fly neurons by their *Drosophila* homologues genes. This has provided molecular confirmation for Cajal's homologies, which he had predicted from pure morphology (Sanes and Zipursky, 2010).

Extensive conservations in 'molecular fingerprints' of particular combinations of transcription factors have also been found between vertebrate and *Drosophila* nerve cord neurons. In addition, mammalian brain hypothalamic neurosecretory cells express the same combinations of transcription factors as their corresponding *Drosophila* or annelid counterparts, which are located within the CNS region traversed by the mouth in protostomes. These neurosecretory peptides, important for sensing and signaling the availability of food, are expressed in the infundibulum of the mammalian brain, through which the gut probably traversed in our hypothetical ancestors (Tessmar-Raible *et al.*, 2007). Thus, *Urbilateria* had a CNS, including eyes, that was sophisticated both from molecular and anatomical standpoints. Before this stage was reached, a long line of Precambrian ancestors must have existed, in which their brains, neural circuits, and eyes were gradually perfected.

3.4. Animals share a conserved genomic tool-kit

Until recently the history of animal life on earth had to be deduced from the fossil record. Rapid advances in DNA sequencing have now made available entire sequenced genomes from multiple animal phyla. Because the genetic code arose only once, evolutionary studies are now less dependent on paleontology. We will be able to reconstruct the history of life on earth, registered in the language of DNA, with a degree of precision that seemed impossible only a decade ago. For those interested on how animal evolution actually took place, comparative genomics offers the best of times.

The most important lesson we have learned so far from genome sequences is that animals from the most diverse phyla share a common ancestral tool-kit of genes (De Robertis, 2008). In particular, all the signaling pathways used by cells to communicate with each other – and therefore to regulate their anatomical position with respect with each other in the body – were already present in pre-bilaterian such as cnidarians (sea anemones,

medusae and Hydra). Therefore, evolutionary changes resulted from the shuffling of a full ancestral set of genes, rather than from the introduction of new genetic mechanisms from scratch. There was remarkably little biochemical novelty during animal evolution.

3.5. Adaptive mutations

DNA sequencing has given us the opportunity of identifying the adaptive mutations that were actually selected during the evolution of animal populations in nature. The main types of variations on which natural selection acted to select the adaptive ones were: cis-regulatory mutations, structural gene mutations, gene duplications and gene deletions.

Cis-regulatory mutations are those found in the regulatory regions – called enhancers – located in cis (in the same DNA molecule) near genes. Enhancers regulate in which tissues genes are expressed. Enhancer DNA sequences provide binding sites for combinations of transcription factors that turn genes on and off. By changing the tissue or region in which a gene is expressed, morphological change can be generated. For example, crustaceans such as shrimp and lobsters evolved a considerable diversity of feeding appendages; it has been shown that these changes repeatedly correlated with independent shifts in the border of expression of Hox genes. New enhancers can be readily generated by bringing together combinations of DNA binding sites. They can also be easily lost without paying a large penalty, because the protein encoded by the gene remains and can still be expressed in other tissues under the control of the remaining enhancer elements. Mutations in tissue-specific enhancers are a major source of variations in evolution (Carroll, 2005). However, because enhancers are not highly conserved in sequence, their mutations are rarely detected by automatic sequence comparisons.

Structural mutations affect the sequence of the proteins encoded by genes. Interestingly, adaptive changes many times result from selection of mutations in the same gene. Melanism can be a useful adaptation. Melanic leopards, jaguars, mice, birds and lizards all arose from amino acid changes that increased the activity of the Melanocortin-1 receptor (Hoekstra and Coyne, 2007). Conversely, decreased activity of this receptor is seen in yellow Labradors and human redheads. Thus, natural selection chooses the same solutions repeatedly.

Gene duplications are very powerful source of evolutionary variation because the duplicated gene can be used to fulfill new functions without loss of the original gene (Ohno, 1970). For the molecular biologist they offer the additional advantage that the duplication – or the deletion – of an

entire gene is easily recognized when comparing genomic DNA sequences, thus facilitating the reconstruction of the history of animals.

Gene deletions are a very effective, although generally underappreciated, source of adaptation. Many cave animals – such as salamanders, shrimp and fish – adapt to their new troglodyte environment by losing their eyes and skin pigment. In the case of Mexican Tetra fish, their entrapment in subterraneous caves has led to deletion events in the *ocular and cutaneous albinism gene-2* that occurred independently in different populations (Protas *et al.*, 2006). Natural selection tends to choose mutations in the same genes over and over again. Although gene deletions are an effective way of rapidly adapting to changes in the environment, this is achieved at the expense of limiting future evolutionary flexibility.

3.6. Gene losses in the ancestral tool-kit

There are 30 bilateral animal phyla with distinct body plans, which can be classified in two branches. In the protostomes (mouth-first), the mouth is formed near the blastopore – these animals include most invertebrates. In the deuterostomes (mouth-second), the blastopore gives rise to the anus and the mouth is perforated secondarily - these animals include the phylum *Chordata* to which we belong. For example, if a gene is found both in fruit flies and in humans, it was also present in their last common ancestor, *Urbilateria*, as well. Similarly, if a gene is found both in pre-bilaterian animals such as sea anemones as well as in humans, it follows that this gene was also present in *Urbilateria*.

The role of gene loss in the evolution of Phyla has been highlighted by the sequencing of a sea anemone genome. The bilaterian lineage separated from cnidarians, at least 650 million years ago, from a common animal ancestor designated *Ureumetazoa*. About 2.5% of sea anemone genes are not present in any higher animals but, interestingly, have homologues in fungi and plants. The human genome contains twenty-plus genes of the Wnt family of growth factors. These can be arranged into 13 subfamilies according to their sequence. The sea anemone has 12 Wnt genes, each corresponding to one of the human subfamilies. (Kusserow *et al.*, 2005). Therefore, *Urbilateria* had genes corresponding to at least 12 Wnt subfamilies. Sequencing of the nematode *C. elegans* showed that it has a grand total of five Wnts; the *Drosophila* genome contains only seven. Thus, our human lineage retained most of the ancestral Wnt genes, while worms and fruit flies lost a great many. There are also examples in the opposite direction, in which humans have lost genes present in other vertebrates such as fish, frog or chick. Comparative genomics indicates that gene losses, as well as duplications, may have played an important role in the evolution of body plans.

3.7. Historical constraints in animal evolution

A key question in Evo-Devo is to what degree the deep homologies in embryonic patterning networks have channeled the outcomes of evolution. Many body plans that could have been excellent functional solutions might not exist in nature because they cannot be constructed unless they are compatible with the developmental networks that control the blueprint of animal body form. The respective contributions of functional needs and structural constraints is of great interest in evolutionary biology (Gould, 2002). Paraphrasing François Jacob, not all that is possible finds its way into the actual animal world.

The deep homologies in the developmental tool-kit seem likely to have constrained animal evolution by natural selection. Constraints resulting from the obligatory use of these ancestral patterning networks should not be considered a negative influence. On the contrary, mutations in these gene networks may have been a positive influence that channeled effective adaptation responses to the strictures of natural selection. Adaptation tends to follow the channel of least resistance to ensure survival of the species and it seems likely that modifications in developmental networks have been used repeatedly to resolve related functional needs. Many anatomical structures now considered to result from convergent evolution may turn out to result from the deep homologies in the genetic structure of all animals. Evolution of animal forms involved tinkering with the conserved A-P, D-V, and other developmental gene networks.

3.8. Open questions in Evo-Devo

Three directions will be particularly important for the young discipline of Evo-Devo:

- First, the reconstruction of the ancestral genetic tool-kit from which all animals were built should be a priority. This is at present a bioinformatic computing challenge. Many complete genome sequences are available already. Ideally the DNA of at least one species for each one of the 34 phyla should be completed. The ancestral tool-kit of yeasts has been determined and has proven interesting. Several groups are close to assembling an ancestral mammalian genome. Reconstructing the hypothetical genome of our urbilaterian ancestors will be very informative concerning the origin of body plans – particularly with respect to the role played by gene duplications and deletions during evolution.
- Second, retracing the adaptive mutations that caused the actual anatomical changes selected by natural selection is another priority. Biology is a historical science, and it will be fascinating to unravel the successive molecular steps by which we evolved into our present human condition.

– Third, determining how cells read their positional information in the embryo and adult tissues within self-regulating fields of cells will have both medical and evolutionary implications. In the organism, cells receive a multitude of signals that must be integrated and transformed into well-defined cell behaviors. These responses include cell division, differentiation and death, and are ultimately the determinants of morphological change.

Conclusion

The merging of Evolution and Development at the end of the 20th century has already provided important insights into how animals evolved an immense variety of body forms. The astonishing realization that has already emerged from Evo-Devo is that all animal life on earth evolved by differential use of the same ancestral tool-kit. A crucial role was played by variations in ancestral developmental gene networks that are hard-wired within our DNA.

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Self-presentation of the New Academicians

Edward M. De Robertis

I was born in Boston, Massachusetts, of Argentinian parents. My mother was a poet and my father a neuroscientist doing postdoctoral training at MIT. At that time they were exiled by dictator General Perón, and therefore in 1950 our family moved to Montevideo, Uruguay. Montevideo in the 1950s was a wonderful place to grow up in.

I attended a grammar and high school run by American Methodist missionaries, which provided a good moral education. There, in kindergarten, I met Ana Marazzi, who at age 15 became my sweetheart and later mother to our three beautiful children. My parents divorced when I was five, but my poet mother provided a wonderful home, and made sure I became a confirmed Catholic.

Medical school in Montevideo offered excellent training in the French tradition. I graduated at age 24. We married the day after my final exam, enjoyed a very brief honeymoon, moved to Buenos Aires, and on the third day began Ph.D. studies in Chemistry at the Institute Leloir in Argentina.

Upon completion of my Ph.D., I was accepted into the lab of the eminent embryologist Sir John Gurdon and shortly thereafter we arrived in Cambridge, England. Gurdon was a wonderful mentor, who taught by example. My debt to him is immense.

After three years as a postdoctoral fellow, and the three more as an independent Scientist in Cambridge, I received a call from the Biozentrum of the University of Basel, Switzerland and became Professor of Cell Biology at age 33. The Director of the Biozentrum at that time was Prof. Werner Arber, who is here today. Thank you, Werner.

In Switzerland we had joint group meetings with the great geneticist Walter Gehring. These were very exciting times, for Gehring's group had discovered a gene sequence conserved in several fruit fly genes that regulated anterior-posterior cell differentiations. We collaborated to determine whether similar sequences might be cloned from vertebrate gene libraries. This resulted in the isolation of the first development-controlling gene from a vertebrate in 1984. The study of these genes, now called Hox genes, opened the door for understanding the genetic control of mammalian development.

Twenty-five years ago, I was offered an endowed Chair of Biological Chemistry at the University of California at Los Angeles. There, we carried

out a systematic dissection of the molecules that mediate embryonic induction in frog embryos. We isolated several genes responsible for the induction of cell differentiation. Most were inhibitors of growth factor signaling and one of them, a protein named Chordin, provided the key to the regulation of dorsal-ventral tissue differentiations, not just in vertebrates, but in all bilateral animals.

Thus, our work contributed to the remarkable current realization that embryonic cell differentiations are controlled by regulatory gene networks common to all animals. These discoveries initiated the young discipline of Evolution and Development, called Evo-Devo for short.

I would like to end on a personal note. I join the discussions of this Academy both as a scientist and as a practicing Catholic. I was therefore deeply touched to receive last year's Christmas card from Bishop Sánchez Sorondo. It started: "*In principio erat Verbum*". Above this, the same passage was written in Greek and one could clearly read that *Verbum* translates as *Logos*. As Pope Benedict XVI reminds us, *Logos* in Greek also means Reason. The next line read: "*Et Verbum caro factum est*". This is of course from St. John's gospel, which is read at the end of every traditional Holy Mass. To believe that the "the Word was made flesh", or *Logos*, or Reason, is not easily achieved. Faith needs nurturing surroundings. I was fortunate to have them during my life.

The Pontifical Academy serves to build bridges between Faith and Science – *Pontifex* means the bridge-builder. Biology, which is my field, has been used as an excuse to create false oppositions between Faith and Reason. I therefore welcome this opportunity to help in your task of building bridges between Science and Faith.

Thank you.