Animal embryogenesis: The conversation of cells

Patrick Lemaire

During the course of embryonic development, a single large cell, the fertilized egg gives rise to a multitude of cells of different types. For embryogenesis to succeed, these cells must be of the right type (photoreceptors, neurons, muscles, skin…), in the right number and in the correct position. Over the past 100 years, developmental biologists have started to decipher the rules used by cells to decide what they should become. They unraveled a sophisticated cell communication system, which we start to understand in quantitative terms, and which this short essay will present.

By essence, the fertilized egg gives rise to all types of cells found in the larva. This is a unique property of the egg (and of some cells, called totipotent stem cells).[1] Most cells in an embryo are restricted in their differentiation capacity and can only give rise to a subset of larval tissues and organs.

Conversely, each larval organ or tissue originates from only a small embryonic territory. By fluorescently labelling individual cells in early embryos and tracking their position and fate at the larval stage, we can draw a "fate map", which associates the position of early cells and larval tissues (Fig. 1).

How does each cell decide which tissue it should contribute? One could imagine two scenarios. First, during the successive divisions of the egg, the fate of a cell could be predetermined by the specific maternal RNAs or proteins located in the portion of egg that it inherits. This imposes strong constraints on the precision of the cell division patterns. Alternatively, all cells could initially be equal, their different destinies resulting from interactions with their neighbors. In this scenario, the precision and reproducibility of the cell division pattern is less crucial.
A simple type of experiment allows developmental biologists to distinguish between the two mechanisms. The experiment consists in extracting a cell (or a group of cells) from its normal embryonic environment, transplanting it elsewhere in a host embryo and analyz-

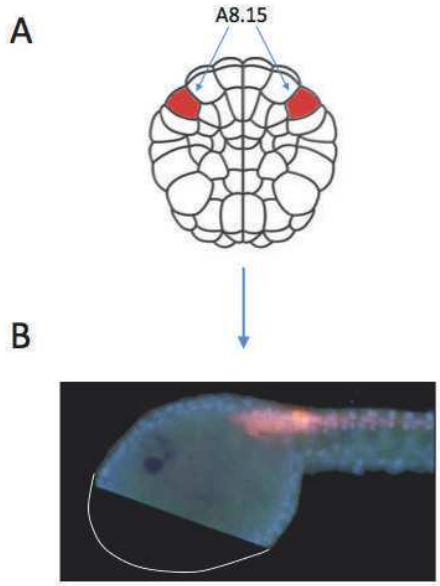

Fig. 1. Tracing the fate of early embryonic cells. A) schematic representation of the vegetal side of an early ascidian embryo (Ciona Intestinalis), when it counts 112-cells, with the position of the left and right A8.15 cells which were labeled with a red fluorescent dye, DiI. B) at the early larval stage, the progeny of the labeled A8.15 cells (red) occupy a posterior part of the central nervous system. Blue: cell nuclei labeled with a DNA dye, DAPI. The approximative outline of the ventral part of the head is indicated with a white line. Image courtesy: Dr. S. Darras, CNRS, Banyuls/mer, France.

ing the fate of the transplanted cells and their neighbors. This type of experiments was carried out in the early 1920s on newt embryos by Hilde Mangold under the supervision of Hans Spemann.[2] They found that the transplantation of a small dorsal territory of early amphibian embryos (coined the Organizer) to a ventral location led to the formation of a second dorsal axis (Fig. 2). In this dorsal axis, the transplanted cells formed the tissues they should normally have formed if they had been left in their original position. Surprisingly, however, the majority of the second axis was formed by cells that should normally have had a ventral fate such as blood. The transplanted cells had thus emitted a signal able to reprogram the fate of their ventral neighbors. These experiments were awarded the 1935 Nobel prize in Physiology or Medicine for the discovery that cell communication was a major organizing force in animal embryos.

Spemann and Mangold's work was followed by decades of frustration and failures to biochemically identify the molecules mediating cell communication. It is only in the 1980s, and the ability to manipulate the activity of specific genes, or to identify by DNA sequencing the genes responsible for developmental defects obtained in genetic screens that the identity of these signals started to be unraveled,[3] causing two major surprises. First, a remarkably small palette of signals, defining even fewer signaling pathways,[4] were used in a reiterated manner to specify the great diversity of cell fates found in animal embryos. Second, most signaling pathways and signals were evolutionarily-conserved and probably already existed more than 600 million years ago in the first animals, despite their apparent simplicity.

But how can the full complexity of cell types[5] in an animal emerge from a language made of just a few words? First, in addition to biochemical communication, cells also sense and react to the mechanical properties of their microenvironment,[6] much in the same way as we react to both touch and words. Mechanosensation can be achieved through the control of the localization within the cell of specific proteins, such as the Yap/Taz transcriptional co-activator. Second, in the same way as words can be assembled into sentences, cells can receive several signals, which are interpreted in a combinatorial manner. Finally, signals are read in a context-dependent manner

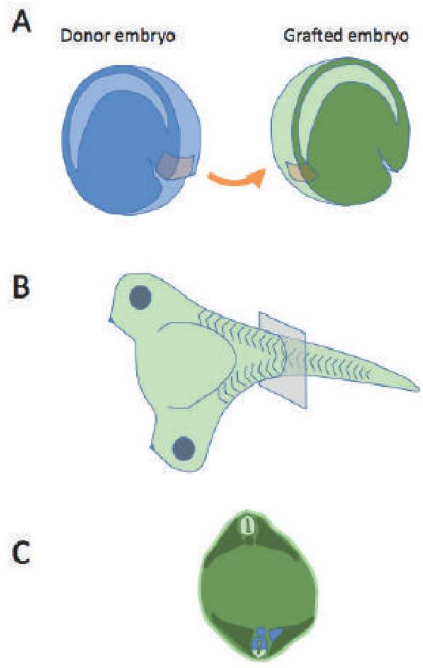

Fig. 2. Organiser transplantation experiment. A) Position of the graft (orange) taken from the dorsal lip of a donor embryo and its placement on the ventral side of a host embryo. The two embryos are at the early gastrula stage. B) at the larval stage, the grafted embryo develops a second dorsal axis, including a fully formed second head. C) Only part of the second axis comes from the graft (blue), the majority of tissues are of host origin and have been reprogrammed by signals from the graft.

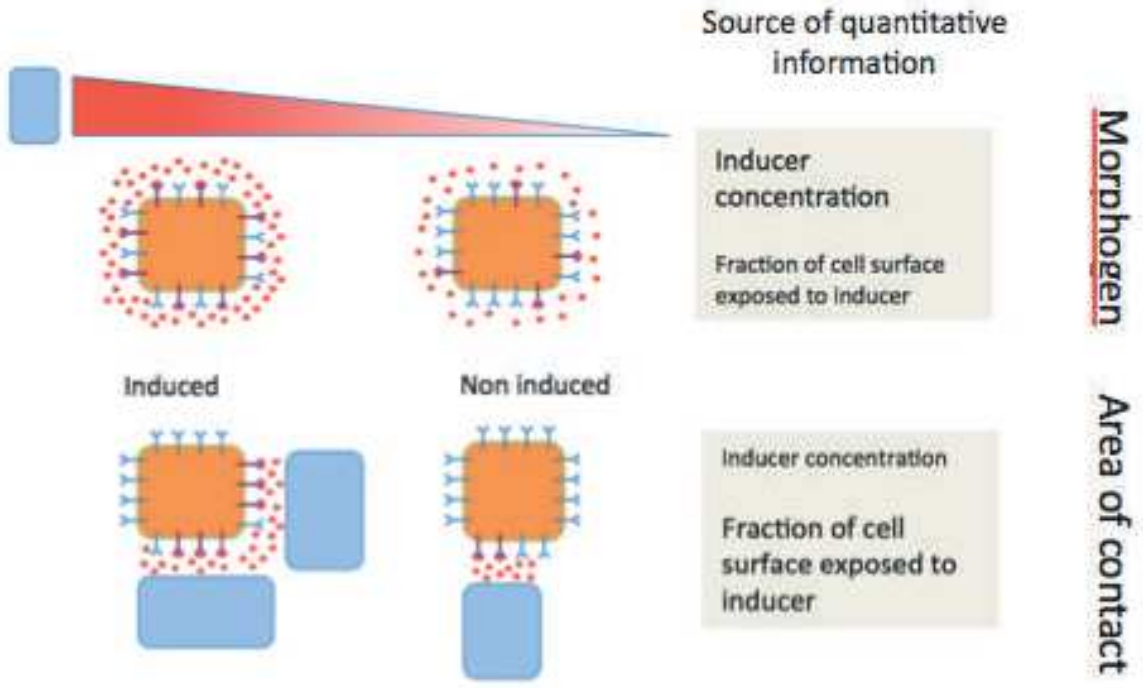

Fig. 3. Morphogen vs contact-dependent inductions. Top, a diffusible morphogen will induce cells according to its concentration, which is defined by the distance to the source. Bottom, when the signal does not diffuse, the fraction of responding cell exposed to the signal can provide the quantitative information needed to decide of the fate of the cell.

that depends on the past history of the cell. For instance, in early ascidian[7] embryos, the differential inheritance of a maternally localized transcription factor, Macho1, by anterior and posterior mesoderm cells explains how the same signal (called FGF) triggers the formation of mesenchyme posteriorly and notochord anteriorly. The human analogy here is that the same words can exist in different languages, but with different meanings. "Sensible" exists in both French and English, but the French word means "sensitive" in English.

Still, all these mechanisms may not suffice to generate the hundreds of thousands of cell types estimated to from a complex adult animal. Unlike in human language, in which a word has the same meaning whether it is whispered or shouted, spoken slowly or rapidly, the intensity and duration of activation of a cell signaling pathway can affect the message it conveys. Applying different concentrations of a signal to a population of pluripotent cells[8] will elicit (scientists also say "induce") different cell fates. Scientists then say the signal acts as a "morphogen" because its quantitative interpretation generates different cell fates. Morphogens are molecules that are secreted from source cells and transported, actively or passively, several cells away from their source, creating concentration gradients. Morphogens thus couple the geometry of the tissue (i.e. the distance to the source) with the generation of the cell types necessary to ensure its function (Fig. 3, top). In such a mechanism, the precision of the local concentration of signal is critical for the outcome of the induction.[9] Of note, not all signals can travel at a distance. Some act by direct contact between the emitting and responding cells. In this case, the quantitative information deciding over the outcome of the induction can be shifted from the concentration of the ligand to the area of contact between source and responding cells (Fig. 3, bottom).

The last thirty years have brought much progress in our understanding of the "language"

used by cells to communicate. Yet, there is still much to discover. Our understanding is currently mostly qualitative. It is only in the past couple of years that single-cell techniques,[10] the next genomic revolution, have given us access to measurements of concentrations large numbers of RNAs or proteins in individual cells rather than large cell populations. There is currently not even a single case in which we understand an induction process sufficiently quantitatively to predict its outcome from the knowledge of the genome and of the precise concentration of active components in the cell.

Finally, one may wonder why it matters to understand the language of cells. Isn't this too esoteric to invest in? Because of the central role of cell communication in the organization of living matter, this curiosity-driven research is intellectually stimulating and pleasing for it seeks to answer a fundamental property of living systems. But there is more to it. First, many of the signaling pathways that control cell fate decisions in the early embryo have been involved in a broad range of human adult diseases, including cancer, osteoporosis, or cardiovascular disease. The fact that they are evolutionarily deeply conserved means that they can be studied in much simpler animal systems than human cells. Second, as R. Feynman wrote on his blackboard in his later years, "What I cannot create, I do not understand". Understanding the language of cells opens the way to building tissues and "organoids"[11] from scratch from naïve pluripotent cells. In the mid-term, such organoids, built from the patient's own cells, could be used in transplantations. The generation of organoids raises ethical issues that need to be carefully addressed. They, however, provide a substitute solution to the situation described in the dystopian novel *Never let me go* by Kazuo Ishiguro, which portrait the life of young men and women raised as a source of organ donations.

[1] While most cells in an animal have the same DNA content, they differ in the cells they can give rise to by division. Totipotent embryonic stem cells have the unique potential to give rise to any cell found in an adult animal when transplanted into an embryo. When totipotent stem cells divide during development, some of their progeny retain their potential, while others gradually lose their totipotency and become pluripotential.

[2] H. MANGOLD, H. Spemann, "Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren", *Roux's Arch. Für Entwicklungsmechanik* 100, 1924, 599-638.

[3] L. WOLPERT, "Do we understand development?", *Science* 266, 1994, 571–572.

[4] A signalling pathway is the succession of proteins used to transmit the information conveyed by an extracellular signal to the nucleus of the cell where DNA, the blueprint of the cell, is located. A typoical plathway will include one or several receptors spanning the outer membrane of the cell, and several protein, some of which translocate to or out of the nucleus in response to a signal.

[5] Our body contains approximately 30 trillion cells, broadly classified into a few hundred cell types. Each of these broad cell types, however, is a mosaic of specialized cells with unique identity and function. There are for instance more than 10 thousand types of neurones in our brain. Characterization of full cell type repertoires in animal embryos and adults require single cell approaches. See note 10.

[6] In a very influential 2006 study published in the Journal Cell, Engler and colleagues showed that stem cells grown on substrates of different elasticity differentiate into different cell types.

[7] Ascidians are marine invertebrates closely related to vertebrates.

[8] Pluripotent cells are cells that can be chemically or mechanically instructed to form different types of cells.

[9] Actually some time, the signal only gives a coarse information, which is subsequently refined by the action of what are called Gene Regulatory Networks. See for example the review by Sagner and Briscoe. A. SAGNER, J. BRISCOE, "Morphogen interpretation: concentration, time, competence, and signaling dynamics", *Developmental Biology* 6(4), 2017, e271.

[10] The ability to study the contents and properties of single cells as opposed to large, often heterogeneous, cohorts of cells. Single-cell approaches are central to identify the full repertoire of different cell types in humans, see the Human cell Atlas project. https://www.humancellatlas.org/

[11] Organoids are biological structures, built in a Petri dish from stem cells, that self-organize into miniature replica of functional organs.