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Early Concepts and Terminology

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KEY POINTS

- This chapter considers the language used within embryological research and how it is evolving.
- The terms used to describe embryos, cells and tissues derive from the social constructs of science during the time they were created.
- Newer terms have been added as scientific methods have increased, although there may not be a consensus on the definition of some terms.
- The application of computer sciences to development has produced its own terminology.
- The use of computer ontologies may impact development and the evolution of embryological concepts and terminology with which to explain the observed processes.

The Changing Concepts and Language of Embryology

Embryological terminology used today is a strange and diverse mixture of terms accrued over the course of two centuries and used as a vernacular language with different dialect depending on the topic and techniques of study. The accumulated terms include:

- The very old concepts generated between 1830 and 1900
- The newer understanding of cell phenotype generated in the 20th century from *in vitro* and *in vivo* experimentation and cell culture
- The very modern and rapidly changing 21st-century terms which describe gene expression and metabolism within embryonic tissues

The language of the latter group is driven by computer ontologies: hierarchies of embryological terms and key words programmed as algorithms, which are used to mine databases and publications. The spur for this interest is a future ability to unlock embryological pathways as a method for treating adult pathology and harnessing the regenerative potential of stem cells.

Origin of the Early Embryological Terms

Embryological terms are a product of their time and a reflection of how developmental science was explained. At a time when the theory of evolution was being formulated in the mid-1800s, Ernst Haeckel promoted a concept which stated that embryos would pass through all the previous evolutionary stages, resembling a series of extant or extinct adult animals as they recapitulated evolution during development.[1] Thus Haeckel designated a *blastula* stage of development when a sphere or bilaminar layer of embryonic cells was present and a later *gastrula* stage achieved after the blastula cells had invaginated to produce more than one or two layers. He also inaugurated the term *gastrulation* to describe the process where cells initially on the embryonic surface move inside the embryo to produce intraembryonic cell populations.

At this time the instruments for examining embryos were rudimentary, and cell theory, being formulated also in the mid-1800s, was still relatively young. Embryologists of the day saw layers of tissue rather than the individual cells composing the layers and did not link the morphology of the earliest cells with differences in function. In studies in which it is clear from the publications that cells could be seen, distinctions among early embryonic cell types probably could not be made with the instruments available. The concepts thus generated by these early embryologists were products of their time, dependent on the methods of experiment and observation customary when they were formulated.

For most of the 20th century, textbooks supported the notion that the tissues of the developed body were derived from one of the 'three germ layers'. Whilst this is not untrue in simplistic terms, the accent on three *layers* (ignoring the cell phenotypes) moved attention away from and limited interrogation of the dynamic differentiation processes occurring in embryos. Without a full range of words with which to think about developmental processes the reflections on, and explanations of, what is seen histologically becomes obfuscated.

A similar interpretive process driven by evolution theory occurred with the description of external embryonic form. In 1828, Von Baer[2] noted that all vertebrate embryos pass through externally similar stages, and Haeckel published a series of drawings demonstrating remarkable similarity between embryos which go on to become very dissimilar adults. This latter concept remained unchallenged for more than a century. Recent examination of Haeckel's pictures, together with a clear analysis of the developmental stages of various organs in each embryo, revealed a different story. Richardson[3] noted that drawings by contemporaries of Haeckel show much more accurate interpretations of mammalian embryos of the same developmental stage with clear differences among them. He noted that Haeckel's drawings had given a misleading view of embryonic development. Thus the idea of one stage of development, during which all vertebrate embryos are the same, promoted extensively at the turn of the 20th century and repeated unchallenged, hampered investigation into what really occurs in a number of vertebrate embryos. This

again obfuscated the search for what is actually present in embryos by limiting the embryological concepts taught, the language used and consequently the expectations and explanations of the processes observed.

Embryology was advanced in the middle and later years of the 20th century by *in vitro* studies of developing reptile, avian and mammalian embryos, particularly using chimeric embryos in which specific cells lines could be followed.[4] These experiments provided information about the similarities and differences among species. Also at this time, the genes expressed within developing tissues were studied, and the range of genes used in basic cell functioning and at particular points of development were elucidated.

The functions of many genes were studied by the experimental production of animals in which specific genes were knocked out or knocked in, and the effects of the homozygous were compared with the wild type. These experiments demonstrated the importance of some genes, with knock-out causing lethality; in other cases, the actual effect of taking one gene out within an embryo was confounded by the catch-up mechanisms in built into development: the change in one part of the system causing compensatory change in the remainder.

Early Embryonic Cell Interactions

Expansion of *in vitro* fertilisation techniques and the selection of healthy embryos for implantation have demonstrated that the secondary oocyte has a range of genes ready for expression to ensure cleavage, morula and blastocyst formation. When the dividing cells are an appropriate size, polarity is expressed, junctions are formed and embryonic cell–cell interaction commences.[5] After hatching from the zona pellucida, the blastocyst is able to interact with maternal tissue. There is no time when the genome is not being read and epigenetic consequences, because of local environmental conditions, are not part of the next process.

Three cell lineages are now identified in the blastocyst, leading to extraembryonic and embryonic cell populations (Table 1.1). All of these lineages express polarity genes and form epithelia.

The process of gastrulation produces cells which do not have an epithelial phenotype (i.e., mesoblast and mesenchymal cells). Recovery of embryonic cells has led to the development of embryonic stem cells which can be immortalised in two-dimensional culture conditions.

Embryonic Cells in Culture

Historically, the definitions of the terms used to describe the putative abilities of embryonic cells were easily found. Today recent papers note the difficulty of accurately defining these terms (Table 1.2). Adult cells can be induced to grow in culture and now so can embryonic cells.

Early *in vitro* culture techniques mainly concerned the growth of adult cells within a two-dimensional physical environment. Cells are grown to confluence and then split into subcultures (passaging) and are regrown many times. The passages and new media promote expansion of the numbers of cells in the culture and prolong the life of the cells beyond that of the original donor. This methodology is still utilised.

Three-dimensional environments, the norm for all body tissues, are being explored in *in vitro* culture. It has been noted that cells in three-dimensional culture systems form organoids, in which the epithelial cells form spheres surrounded by

TABLE 1.1 Lineage Concepts

Term	Meaning
Three cell lineages identified as zygote undergoes cleavage	• Trophectoderm—will become placenta and extraembryonic membranes • Hypoblast—sometimes called primitive endoderm; maintains the primitive streak • Inner cell mass—sometimes called primitive ectoderm; usually termed epiblast; all embryonic cell lines are derived from this lineage
Polarity genes	Cells within an early embryo form epithelia and mesenchyme. The epithelial cells exhibit polarity genes which specify the apical, basal and lateral surfaces; the position of junctional complexes; and the direction of the mitotic spindle during cell division. Not observed before compaction in morula.[6]
Germ layers	These were historically the *ectoderm, endoderm* and *mesoderm*. The terms are still widely used regardless of cell phenotype. They all derive from the epiblast of the early blastocyst.

TABLE 1.2 Definitions of the Terms Used to Describe Zygotes and Stem Cells

Term	Meaning
Totipotent	The ability of a single cell to develop into an adult organism and generate offspring. In humans, the zygote is totipotent. The loss of totipotency is now seen as a process.
Pluripotent	The ability of a single cell to develop into cells from one of the 'three germ layers' and 'germ' cells *in vitro* and *in vivo*. EpiSCs are postimplantation epiblast stem cells.
Embryonic stem cells	Human embryonic stem cells (hESCs). Origin not clear. Not quite the same as inner cell mass cells. Need specific culture conditions; grow in two dimensions. Now have been adapted to long-term *in vitro* culture.
Human-induced pluripotent stem cells (hiPSCs)	Cells derived from adult cells (e.g., fibroblasts) which have been cultured with specific transcription factors. They undergo transition to epithelial cells and express epithelial genes, becoming polarised. They also change their metabolism.
Human spheroids or organoids	When hiPSCs are grown in three-dimensional culture and encouraged along a particular developmental pathway, they form spheres of inner epithelial and outer mesenchymal phenotypes. Organoids have been created from hiPSCs specified as endoderm which differentiate into airway or gut phenotypes with appropriate epithelial and supporting mesenchymal cells. Self-organisation into layered tissues has also been seen in three-dimensional cultured brain cortical cells and retinal tissue.

mesenchymal cells.[7,8] These self-organising cell lines have been implanted into animals and will continue growing.[9] The ultimate aim of these studies is to grow replacement portions of gut, respiratory conducting airways or kidney which ultimately can be used for transplant.

Cultured cells have also been purposefully arranged in specified three-dimensional shapes by bioprinting methods and encouraged to grow and differentiate along specific lines by the addition of targeted growth factors to the media.[10] The complexity of setting up all three-dimensional systems and recording cell growth, movement and interaction are particularly challenging.

Further experimental methods have attempted to immortalise embryonic cell lines. By specifying the growth factors used in the culture media and the oxygen levels supplied and by forcing cells to change their phenotype (mesenchyme to epithelial), the cells have been driven along particular developmental pathways to form specific cell lines, e.g., cardiac myocytes, hepatocytes and neurons.[11,12] Such cultures are used for further optimisation of culture conditions, to gain knowledge of the genes the cells are utilising, and for testing drugs on cells in culture rather than on laboratory animal species.

Interpretation of the Genome

The latter years of the 20th century and the beginning of the 21st saw an explosion of interest in embryological pathways, first because of the identification of the human genome and the genomes of the commonly used laboratory animals (Table 1.3) and second because it was thought that re-expression of embryonic genes and pathways in an adult could lead to treatment of many pathological conditions.

The success of the Human Genome Project led to elucidation of the genomes of laboratory species and greater understanding of the shared genes upregulated in development. Information on the temporal and spatial regions of gene expression during development superimposed on internal and external embryonic form has been shared via the internet. Such websites also have the methodologies for demonstrating specific genes and transcription factors.

TABLE 1.3 Internet Sources of Information on a Range of Genomes

Genome and proteins	Internet site
Human Genome	National Human Genome Research Institute Has timeline for the Human Genome Project
Human Proteins	• Human Proteome Project; Human Proteome Map • Human Protein Atlas Human Developmental Studies Network (HUDSEN) • Electronic atlas of a developing human brain • Human spatial gene expression database • Virtual Human Embryo • Multidimensional Human Embryo Both use Carnegie staging for human embryos Brainspan Atlas of the developing human brain, developmental transcription factors
FlyBase	A database of the genome and genes expressed in *Drosophila* throughout development
WormBase	A database of the genome and genes expressed *Caenorhabditis elegans* and other species
Mouse eMAP	• Edinburgh Mouse Atlas Project • eMA: three-dimensional mouse anatomy atlas • eHistology, with serial sections of mouse embryos throughout development • eMAGE: gene expression database for mouse embryos DBTMEE • Database of transcriptome in mouse early embryos
echickatlas	• Three-dimensional anatomical atlas of chick embryo development • e-Chick Atlas of gene expression
Geisha	Gallus Expression In Situ Hybridisation Analysis • Anatomical atlases • Chick development stage series • Bird genomes • Transgenic lines of chickens and quail

Computing Sciences and Embryological Terminology

Embryology has now become a domain of computer sciences as well as laboratory-based sciences. Powerful computing was necessary for the collation of the genomes and the proteins encoded. Two interrelated lines of research can now be noted: (1) the relationships among cells, tissues, organs and time within developing embryos and (2) the relationship between genes and the molecules they encode. Ontologies, hierarchical structures of specified vocabulary, have been created for developmental processes; embryonic cells and tissues; for genes, transcription factors and proteins. New genes, or the spatial and temporal expression of known genes, are added to specific ontologies either by a curator or by the computer ontology algorithm itself (an inferred electronic annotation). Predictions of future gene function can be gained from these methods.

The laboratory techniques of mass spectrometry and microarray methods can identify proteins and metabolites in very small samples of culture media. Information concerning what genes the cells are expressing and what proteins they are making at each time point of development has been shown to provide, for example, objective data in the assessment of preimplantation embryos from *in vitro* fertilisation.[13]

These methods have also been used to analyse the supernatant of embryo cultures at specific time points or to analyse the supernatant of induced pluripotential stem cells culture as the cells are induced to follow specific phenotypic pathways (Table 1.4). The techniques of high-resolution mass spectrometry are now able to identify and quantify protein in single embryonic cells.[16] The language used to describe these techniques is now part of the embryological vocabulary.

The use of powerful computing has also enabled three-dimensional images of animal and human embryos of all stages to be made available, showing external form and serial histological sections. The spatial and temporal location of specific gene expression may also be added to these images (see Table 1.3).