



Early Development I: Cleavage

Four Major Developmental Events

There are 4 major developmental processes that occur in human embryogenesis.

- **Cell Division (Cleavage)** - Converts 1 cell to many; the egg is one cell, the embryo is multicellular; the conversion from one cell into many involves an initial cleavage (embryonic mitoses) phase followed by regular mitotic cell divisions.
- **Cell Differentiation** - formation of different, specialized cell types; the egg is one cell type, the embryo contains hundreds of cell types; understanding how cells specialize is a fundamental problem of developmental biology
- **Morphogenetic Events** - literally the "generation of shape", morphogenesis results in the embryonic organization, the pattern & polarity to cells, organs & tissues; the egg is round while the embryo has a specific organization of multiple layers of different cells; the terms morphogenesis and differentiation are not synonymous although many researchers today make this mistake in terminology; cell differentiation is just one component of morphogenesis
- **Growth** - Increases size of organism; the embryo increases dramatically in size from the next to invisible egg to the full grown fetus.

Of course there are other component events that also occur such as apoptosis (controlled cell death) which underlies many morphogenetic events in the formation of tissues and organs. Apoptosis was discussed earlier in the course.

The Timing of Early Development

As we work through early development over the next several lectures you should have a basic idea of when specific events occur. These are shown in the following figure. Note that many of the events overlap (i.e., one event doesn't have to finish before the next begins).

Development: The First 35 Days	
Days 0-7	Fertilization → Cleavage → Blastocyst Formation
Days 8-14	Implantation
Days 15-21	Gastrulation; Somites begin to form; vascular systems starts to develop
Days 22-28	Neurulation; Neural crest migration begins; Somites differentiate; embryo folds; lungs, guts, Urogenital system and Heart start to develop; Arm limb bud appears; Ears start to develop
Days 28-35	Nervous systems develops; Eyes start to develop; leg limb bud appears; Spinal column and ribs begin to form; Peripheral nervous system develops; Stomach forms; Indifferent external genitalia begin to develop as male or female

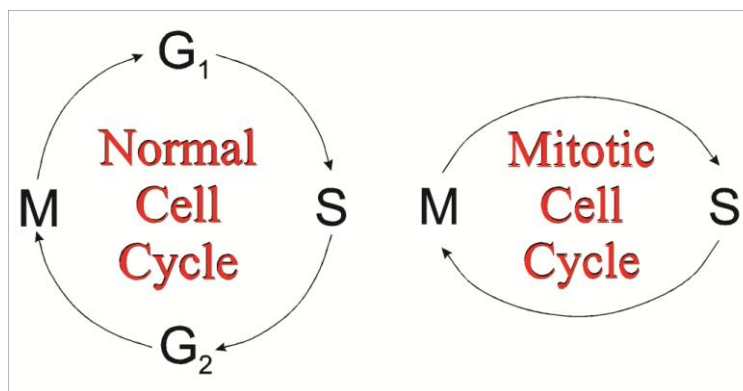
Cleavage

Here we will focus on the events that occur during the first 7 days after fertilization. This will take us to the hatched blastocyst which will have arrived at the uterus which will be ready to accept it for implantation.

Cleavage: Functions & Events

- Initial divisions of zygote to form the multicellular embryo
- During cleavage the cells are called "Blastomeres"
- They are special mitotic divisions
- Since they are mitotic divisions, they maintain the 2N complement
- They are rapid cell divisions with no intervening growth (G₁ & G₂) phases
- Cells become smaller with time
- Holoblastic Cleavage: cells are completely separate

Mitotic vs. Normal Cell Cycle



- Cleavage is characterized by a shortened cell cycle
- During cleavage G₁ and G₂ stages are by-passed so cells simply progress from S (DNA synthesis) to M (mitosis) without the intervening growth phases
- As a result cleavage cells continue to decrease in size until they approximate the size of somatic cells
- At the blastocyst stage the transition occurs to a normal cell cycle: G₁, S, G₂, M.

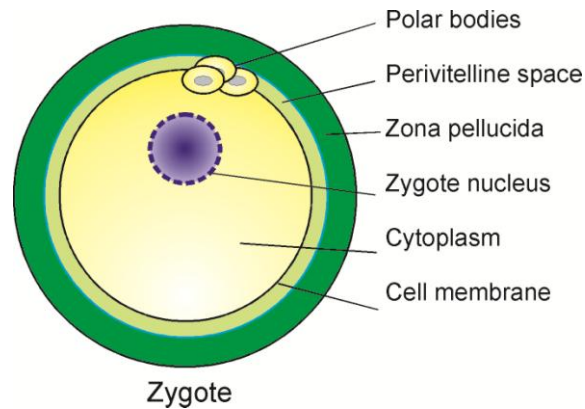
The Purposes of Cleavage

- Converts unicellular zygote to multicellular embryo
- Produces many cells that can interact and be moved around
- Maintains diploid complement of cells--all are genetically identical
- Human cleavage is not synchronous; all of the cells do not cleave at precisely the same time, as a result embryos with odd numbers of cells can be seen at various times
- Slow cleavage; takes approximately 12-24h between each cell division
- No growth occurs during early cleavage, so the total embryo will remain ~100 microns (0.1mm) in diameter

The Stages of Early Cleavage

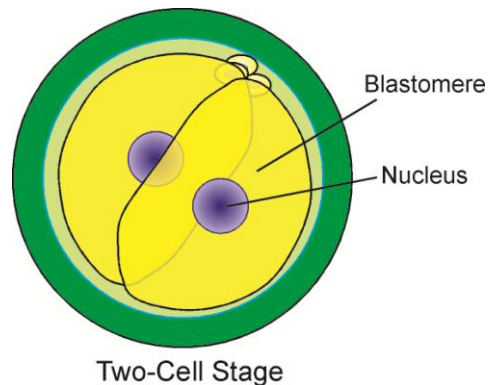
- Cleavage begins about 24h after the egg has been fertilized once the pronuclei have fused.
- We will discuss cleavage as if each division occurs synchronously because it is easier to understand this way

Cleavage



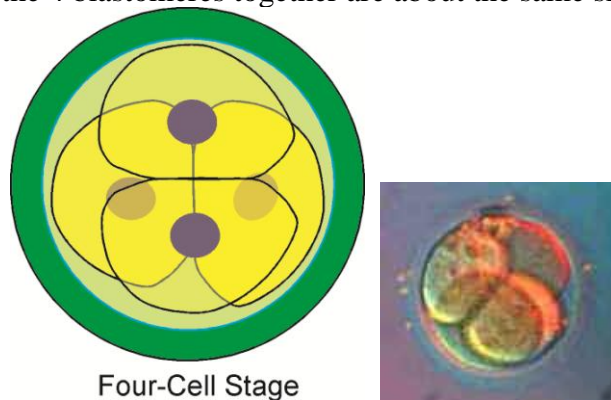
1st Cleavage

- Resulting embryo is 2 cells (i.e., 2 blastomeres or 2 cleavage cells)
- DNA has been synthesized: Embryo has double the DNA content of zygote
- Embryo has made membrane to surround both cells
- Growth has not occurred so the 2 blastomeres together are approximately the same size as the original zygote



2nd Cleavage

- Embryo is 4 cells
- DNA has been synthesized: Embryo now has 4x DNA content
- Embryo has made membrane to surround all four cells
- Growth has not occurred so the 4 blastomeres together are about the same size as the original zygote



3rd Cleavage

- Embryo is 8 cells

Cleavage

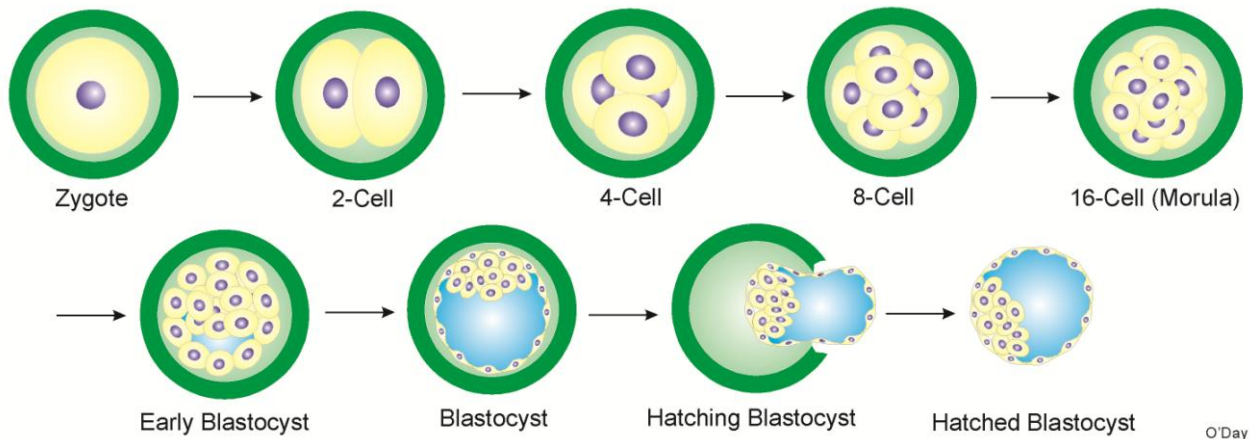
- More DNA has been synthesized: Embryo now has 8x DNA content
- Embryo has made membrane to surround all 8 cells
- DNA duplication and membrane synthesis will continue as cells continue to divide
- Growth has not occurred so the 8 blastomeres together are approximately the same size as the zygote
- Embryo will now undergo compaction just prior to next division leading to 16 cell stage



Cleavage, Compaction, Blastocyst Formation and Hatching

The following diagram takes the human embryo from the zygote surrounded by the zona pellucida to the hatched blastocyst stage where it is ready for implantation in the uterus. As cleavage continues (compaction is not shown), fluid appears in between the cells after the morula stage resulting in the early blastocyst. This fluid accumulates in an increasingly larger cavity called the blastocoel as differences in the cell populations become evident. The blastocyst hatches from the zona pellucida preparing it for implantation.

Early Embryonic Development



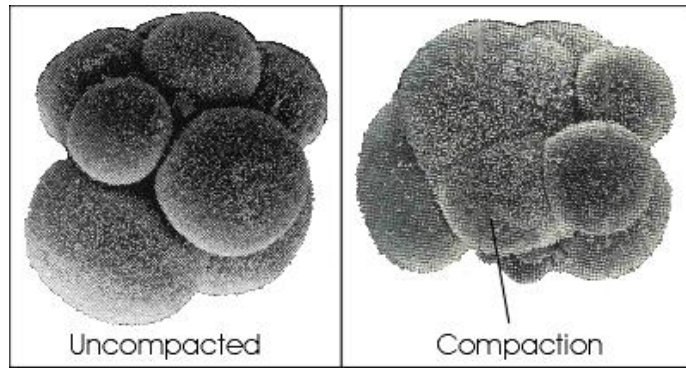
Compaction

- At the 8 cell stage the human embryo undergoes a process called compaction.
- It results from cells adhering together more tightly
- The embryo becomes more compact, but the cells still remain separate from each other
- The cell adhesion protein, E-cadherin appears at the time of compaction and causes the cells to adhere together more tightly than previously.

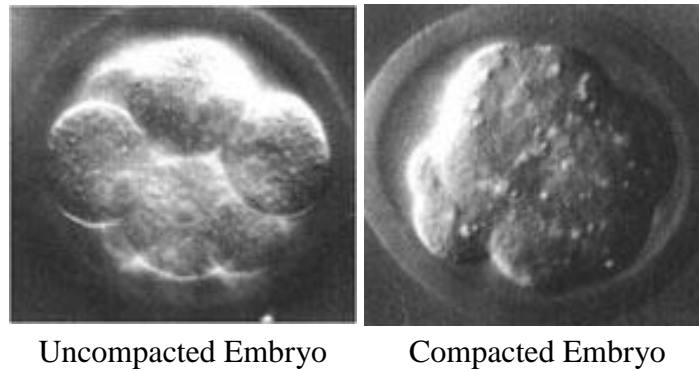
Mammalian compaction

Scanning Electron Microscope image

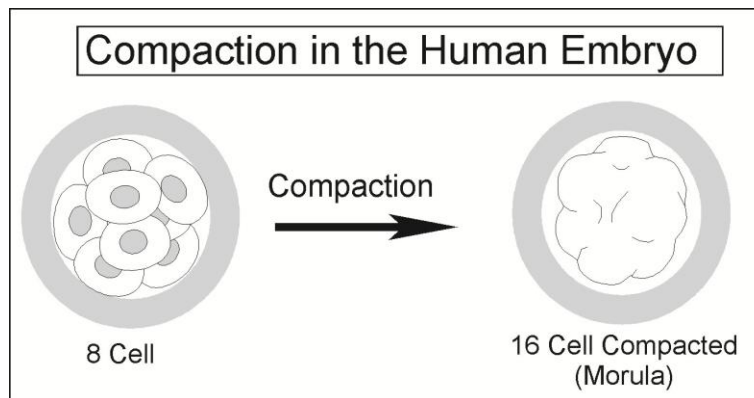
Cleavage



Phase Contrast Microscope image

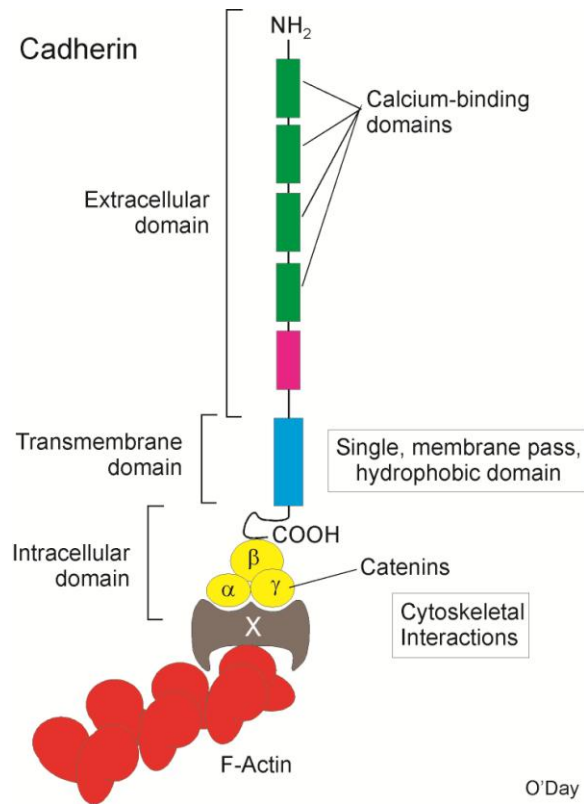


The following diagram shows the process of compaction.



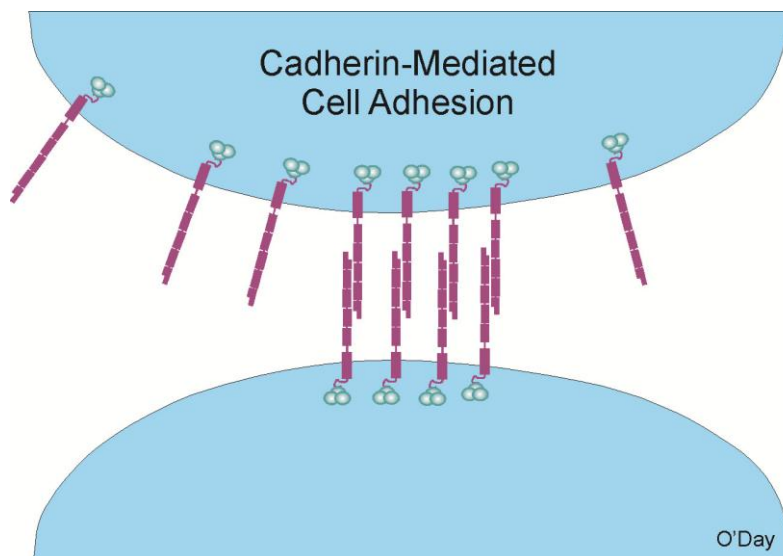
What is E-Cadherin?

Cleavage



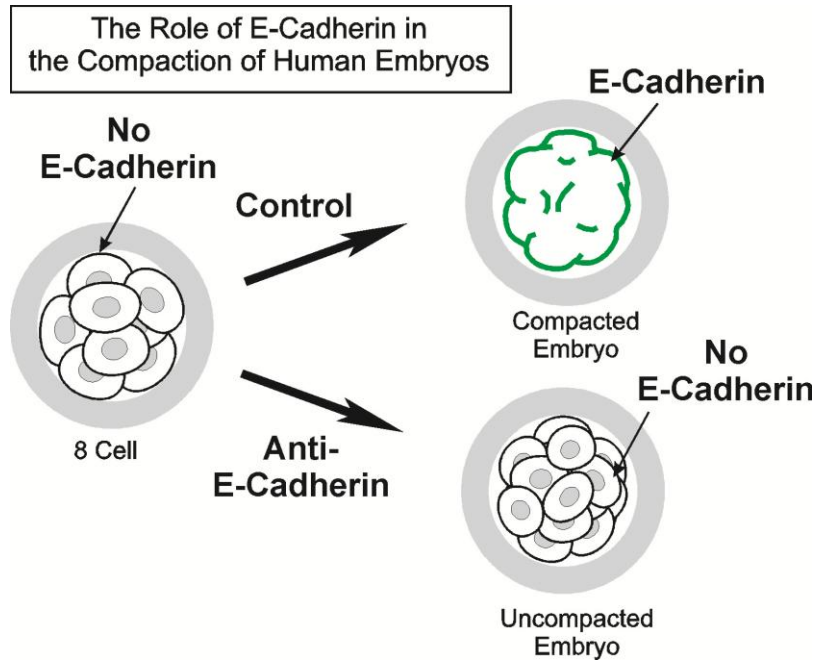
- Ca²⁺-dependent cell adhesion molecule
- Homophilic (cadherin-cadherin) binding
- Catenins mediate binding to cytoskeleton
- E-Cadherin (Uvomorulin) is present in early embryonic development; replaced by N-Cadherin at gastrulation

Here's how cadherin interacts homophilically (same molecules bind together) to cause cell adhesion:



Cadherin & Compaction

As demonstrated by the following experimental results, the appearance of cadherin has been detected at the surfaces of cells of compacted embryos using immunofluorescence microscopy with cells stained with anti-cadherin antibodies (green staining). If the cells are treated with anti-cadherin prior to compaction, they do not undergo compaction. This occurs because the anti-cadherin binds to any cadherin molecules that appear preventing them from being detected or from binding to other cadherin molecules. This work reveals the importance of cadherin in the process.



As the essential role of cadherin in the compaction of the human embryo continues to be analyzed, new molecular components are also being identified as part of the process. Epithin is a mammalian transmembrane serine protease that has been found to be associated with E-cadherin in a diversity of tissues. As the following figure shows, epithin and E-cadherin co-localize at both the 8-cell and morula stages.

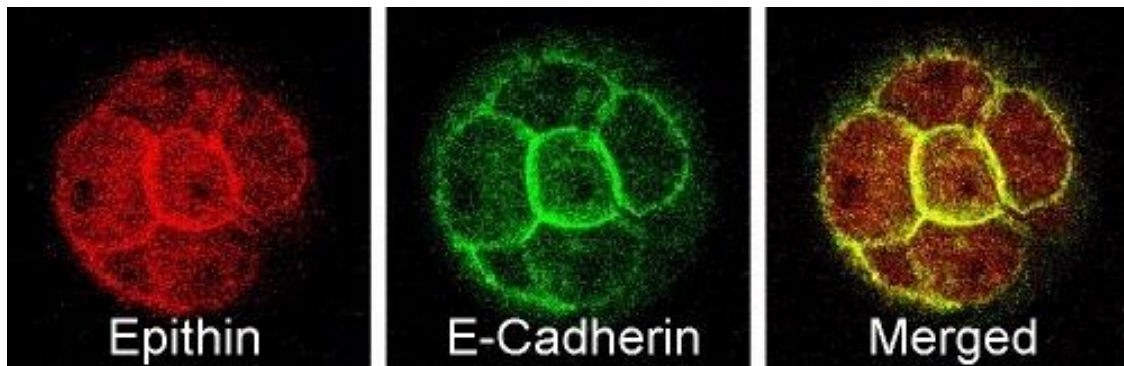
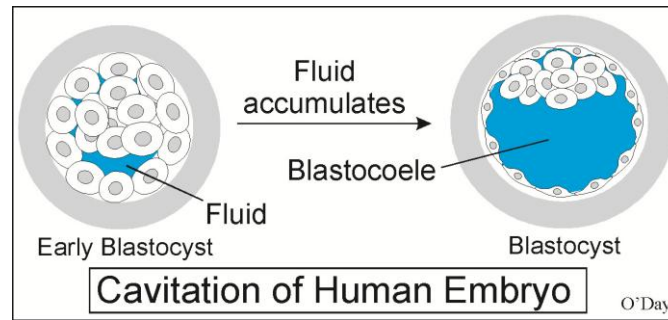


Figure Source: Khang et al, 2005. *Developmental Biology* 281 : 134-144 ; modified from Figure 3, A-C; pg. 139)

This co-localization is suggestive but more insight was gained from insight with the use of RNA interference (RNAi). Embryos in which epithin gene expression was silenced by RNAi failed to express epithin at the cell surface, did not compact and stopped development at the 8-cell stage indicating that epithin is essential for compaction.

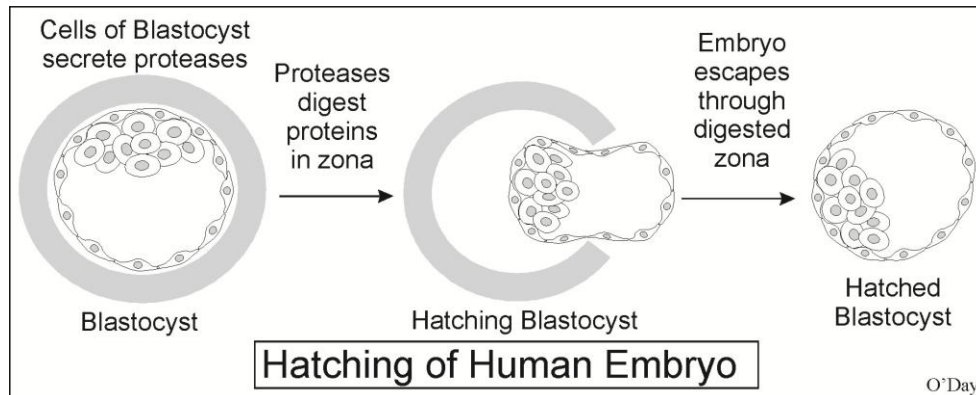
Formation of the Blastocyst

Cleavage



- Fluid begins to appear between blastomeres
- Process is called "Cavitation"
- Begins ~4 Days after fertilization
- Produces "Blastocoele": the fluid-filled cavity of blastocyst

Hatching of the Blastocyst



- Hatching occurs just prior to implantation
- Embryo breaks through ZP due to proteases secreted by blastocyst
- Inability to hatch is a reason for infertility; could be due to altered zona pellucida or to the absence of essential protease for zona digestion
- Often assisted hatching is done *in vitro* during ART procedures
- In mutant mouse embryos lacking ZP1, the zona pellucida is weak and the embryos hatch prematurely.

References

Khang et al, 2005. Expression of epithin in mouse preimplantation development: Its functional role in compaction. *Dev. Biol.* 281: 134-144

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