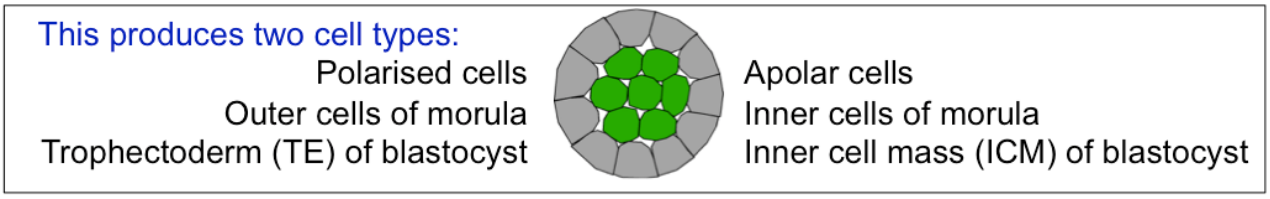
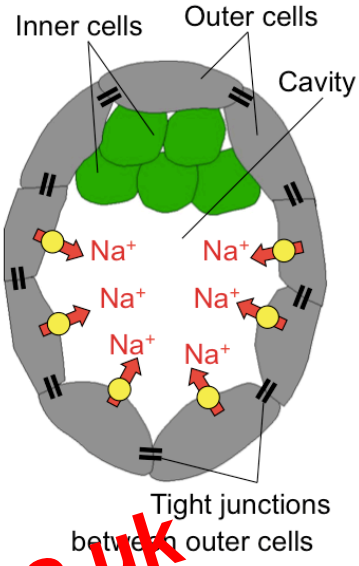
This therefore produces two cell types, as shown below.

- Polarised cells, forming the outer cells of the morula and the trophoectoderm cells of the blastocyst
- Apolar cells, forming the inner cells of the morula and the inner cell mass (ICM) of the blastocyst



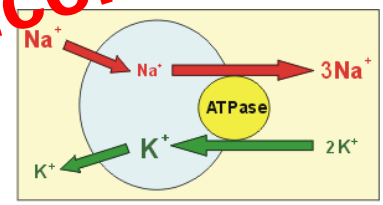


Blastocyst formation

The morula, which precedes the blastocyst, is an early embryo composed of 16 **undifferentiated** cells. Shortly following the morula's entry into the uterus from the Fallopian tube, the morula becomes the blastocyst through **cellular differentiation** and **cavitation.** The morula's cells **differentiate** into two types: an inner cell mass (ICM) growing on the interior of the blastocoel and trophoblast cells growing on the exterior.

The **animal pole** refers to the side of the blastocyst where the ICM resides, and the **vegetal pole** is on the opposite side.

Cavitation is the process by which a fluid cavity forms inside the embryo. The trophoblast cells pump sodium ions into the centre of the embryo, which causes water to enter through **osmosis.** This forms an internal fluid-filled cavity called the **blastocoel.** This distinguishable blastocoel cavity, in addition to cellular specification, are both hallmark identities of the blastocyst.



Therefore, blastocyst formation uses an **energy (ATP)-dependent pump system** where the **Na^+/K^+-ATPase** pumps Na^+ out of the cell and K^+ into the cell. These pumps are formed on the **basal** (inner) surface of the outer cells and allow Na^+ to be pumped into the blastocyst cavity and subsequently **water** is drawn into the cavity by osmosis to cause it to **expand**. The tight junctions ensure there is **no leakage**. The fluid between the cells eventually coalesces and forms one large fluid-filled cavity called the **blastocoel**. The inner cells remain at one end (as the ICM). After this has occurred, the **morula becomes a blastocyst.**

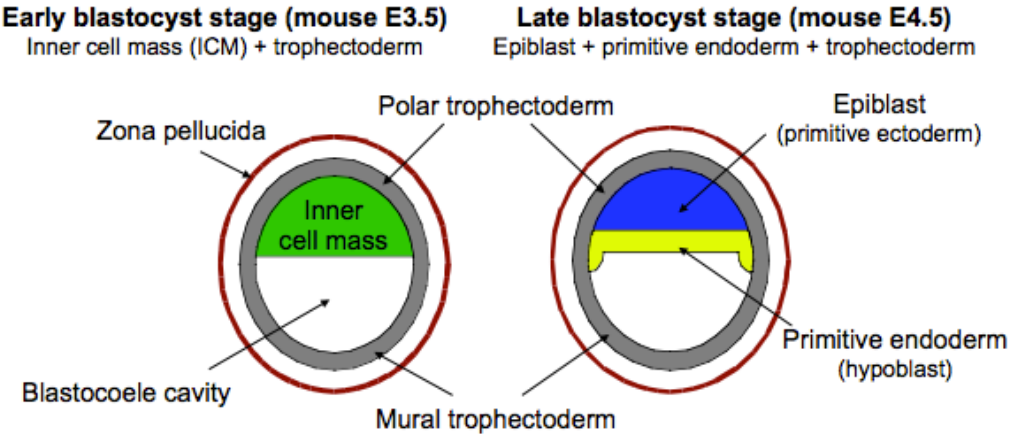
From morula to blastocyst stage, this involves the production of ATPase pumps on the basolateral face of these polarized cells. Na pumped from the cell into the ECM allows a change in osmotic pressure, thus favouring water movement following Na. This produces a fluid bubble in the middle of the embryo called the blastocoel. The zona pellucida surrounds the embryo here, although not shown.

Cell lineages in the late blastocyst stage

There are **three** primary lineages established at the late blastocyst stage: the **epiblast**, **primitive endoderm** and **trophectoderm**, all three of which produce some extraembryonic structures.

Large nuclei form in the **mural** trophectoderm cells, with cells here stopping proliferation but continuing DNA replication, hence the large size of the cells. **Polar** trophoectoderm continue to **proliferate** (note the term 'polar' is used in a different contex referring to these cells being at the pole of the cell).

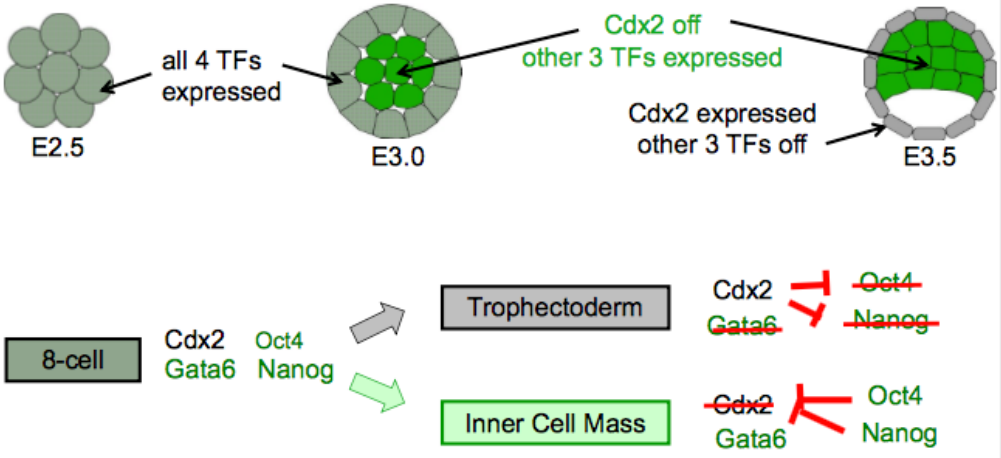
The **epiblast** and **primitive endoderm** group of cells have different developmental functions whereby the epiblast produces the entire fetus. In rodents, there are further layers – e.g. the parietal endoderm etc.



It has since been shown via imaging that the mitotic spindle orientation is **not strictly regulated**; and cells can divide in different orientations and still go on to generate inner and outer cells. **Cell shape and mechanical forces** play a key role in ensuring the final disposition of inner and outer cells, regardless of initial location after cell division. This sorting mechanism reflects **physical properties** rather than a commitment to a TE or ICM fate.

It is the **formation of an outer polarized epithelium** that ensures the development of an increasingly stable inside environment in the embryo, allowing **differential signalling** dependent on cell position. Recent evidence that the **Hippo signalling pathway** plays a key role in driving ICM versus TE fate provides the potential mechanism to link the position and polarity hypotheses.

In summary, within the first lineage decision (TE vs ICM), Cdx2 expression is restricted to trophectoderm, as summarized to the right, with the **Hippo signalling pathway** plaing an important role in allowing Cdx2 to become restricted to TE.



Hippo signalling pathway

The Hippo signalling pathway regulates **organ size** via regulating transcription factors that control cell proliferation and apoptosis (although this is rare in preimplantation embryos). *Drosophila* Hippo mutants show **tissue overgrowth**, giving a hippopotamus-like phenotype.

The Hippo pathway is a **kinase (phosphorylation) cascade** which changes protein activity (as phosphorylation results in switching off Cdx2). The Hippo pathway plays an important role in **restricting Cdx2** to the outer cells.

Yap and Tead4 are needed for Cdx2 expression. Expression of *Cdx2* and formation of the TE is absolutely dependent on the transcription factor **Tead4**, and its coactivator partner, **Yap1**. (Therefore, Yap is a transcriptional co-activator, and Tead4 a transcription factor). Unphosphorylated Yap enters the nucleus and binds to Tead4.

Although Tead4 and Yap1 are expressed in all of the cells of the preimplantation embryo, Yap1 is only localized to the nucleus in the developing outer cells, overlapping with Cdx2 expression. Yap1 is phosphorylated by the activity of the Hippo signalling pathway kinase **Lats1/2** in the inner cells, leading to Yap1 cytoplasmic sequestration and hence absence of Tead4 activity in inner cells. **Therefore, an active Hippo pathway phosphorylates Yap which prevents it entering the nucleus, and thus Cdx2 is not expressed.**

Cdx2 expression depends on **Tead4 and unphosphorylated Yap** as shown to the right.

The posttranscriptional method of ensuring the restricted expression of Cdx2 can explain that initiation of cell fate specification leading up to the blastocyst. The inhibition of TE versus ICM by the reciprocal action of Cdx2 and Oct4 at the blastocyst stage provides the means of ensuring ongoing lineage restriction.