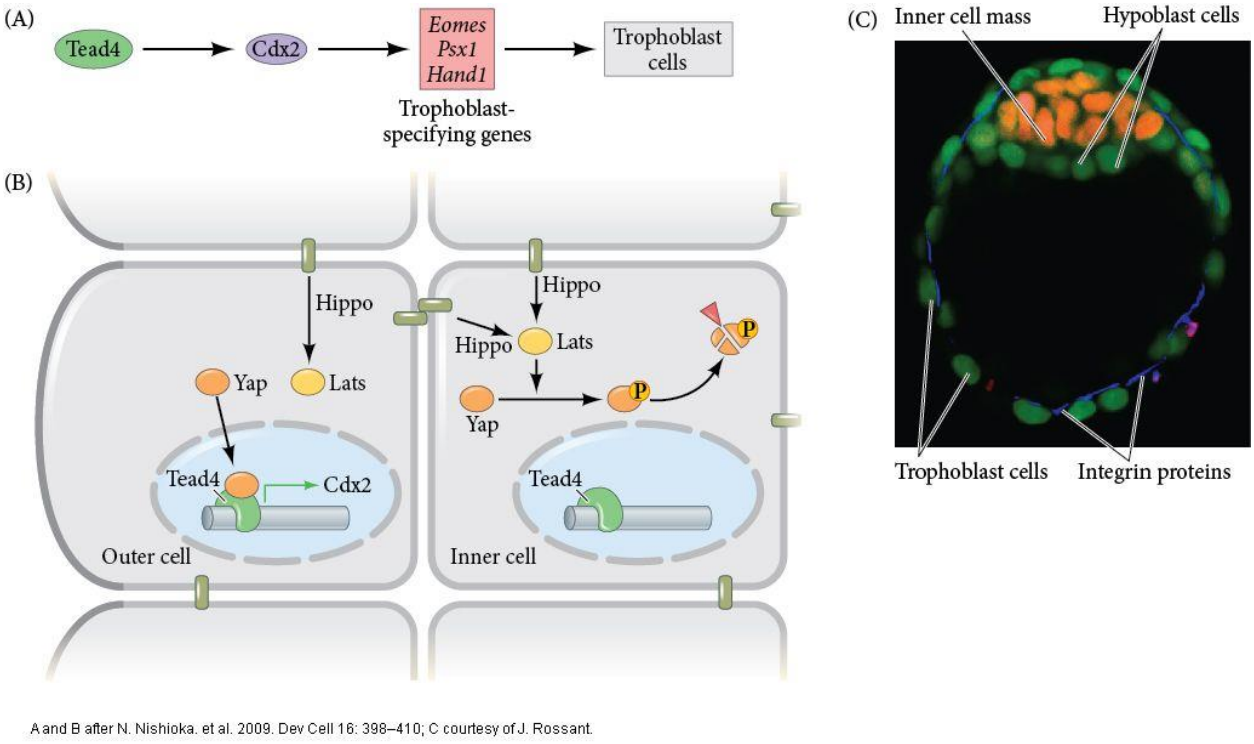
<Further Development 13_04>

The Role of Hippo Signaling during Trophoblast-ICM Determination

The trophoblast is specified on the external cells, while the inner cell mass will form the embryo and amnion. The Hippo pathway is critical in this process (Yildirim et al 2021). Only trophoblast cells synthesize the transcription factor Cdx2, which downregulates Oct4 and Nanog (Strumpf et al. 2005). The activation of the *Cdx2* gene in the trophoblast cells appears to be regulated by the Yap protein, which in turn is a co-factor for the transcription factor Tead4 (**Figure 1A**). Tead4 is found in the nuclei of both the cells that will become the inner cell mass and the outer cells that will become the trophoblast, but it is activated by Yap only in the outer compartment. That is because Yap can enter the nucleus in the outer cells and thereby allow Tead4 to transcribe trophoblast-specifying genes such as *Cdx2* and *eomesodermin* (*Eomes*). In contrast, the inner cells, with each of their surfaces surrounded by other cells, activate the gene for Lats, a protein kinase that phosphorylates Yap (**Figure 1B**). Phosphorylated Yap cannot enter the nucleus and is degraded (Nishioka et al. 2009). Therefore, in the inner cells, Tead4 cannot function and *Cdx2* remains untranscribed (see Wu and Scholer 2016). Cdx2 blocks the expression of Oct4, and Oct4 blocks the expression of Cdx2. In this way, the two lineages become separated. (For more, see Chapter 5, Figure 5.10.)



A and B after N. Nishioka, et al. 2009. Dev Cell 16: 398–410; C courtesy of J. Rossant.

Figure 1 Possible pathway initiating the distinction between inner cell mass and trophoblast. (A) The Tead4 transcription factor, when active, promotes transcription of the *Cdx2* gene. Together, the Tead4 and Cdx2 transcription factors activate the genes that specify the outer cells to become the trophoblast. (B) Model for Tead4 activation. In the outer cells, the lack of cells surrounding the embryo sends a signal (as yet unknown) that blocks the Hippo pathway from activating the Lats protein. In the absence of functional Lats, the Yap transcriptional co-factor can bind with Tead4 to activate the *Cdx2* gene. In

the inner cells, the Hippo pathway is active and the Lats kinase phosphorylates the Yap transcriptional co-activator. The phosphorylated form of Yap does not enter the nucleus and is targeted for degradation. (C) Mouse blastocyst in which the Oct4 protein in the inner cell mass is stained orange. The extracellular lineages (trophoblast and hypoblast) are stained green.

Literature Cited

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