

## CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor***Specifying the Pancreatic Islet through Biomechanical Forces**

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How do living cells respond to physical forces, including those relayed from the complex cellular and extracellular microenvironment within a tissue? We know that these forces affect nearly every facet of cellular biology and function, and yet we understand little about the underlying mechanisms. A study recently reported by Mamidi and colleagues<sup>1</sup> helps to fill this gap. They examined developing pancreatic cells and found that changes in the extracellular milieu trigger intracellular cytoskeletal dynamics and transcriptional changes that profoundly affect the “decision” by cells to differentiate into islet endocrine cells. By manipulating the culture conditions of human embryonic stem cells, as well as by using gain- and loss-of-function approaches both *in vivo* and *in vitro*, the authors showed that biomechanical signaling pathways drive cell-fate decisions in pancreatic cell types.

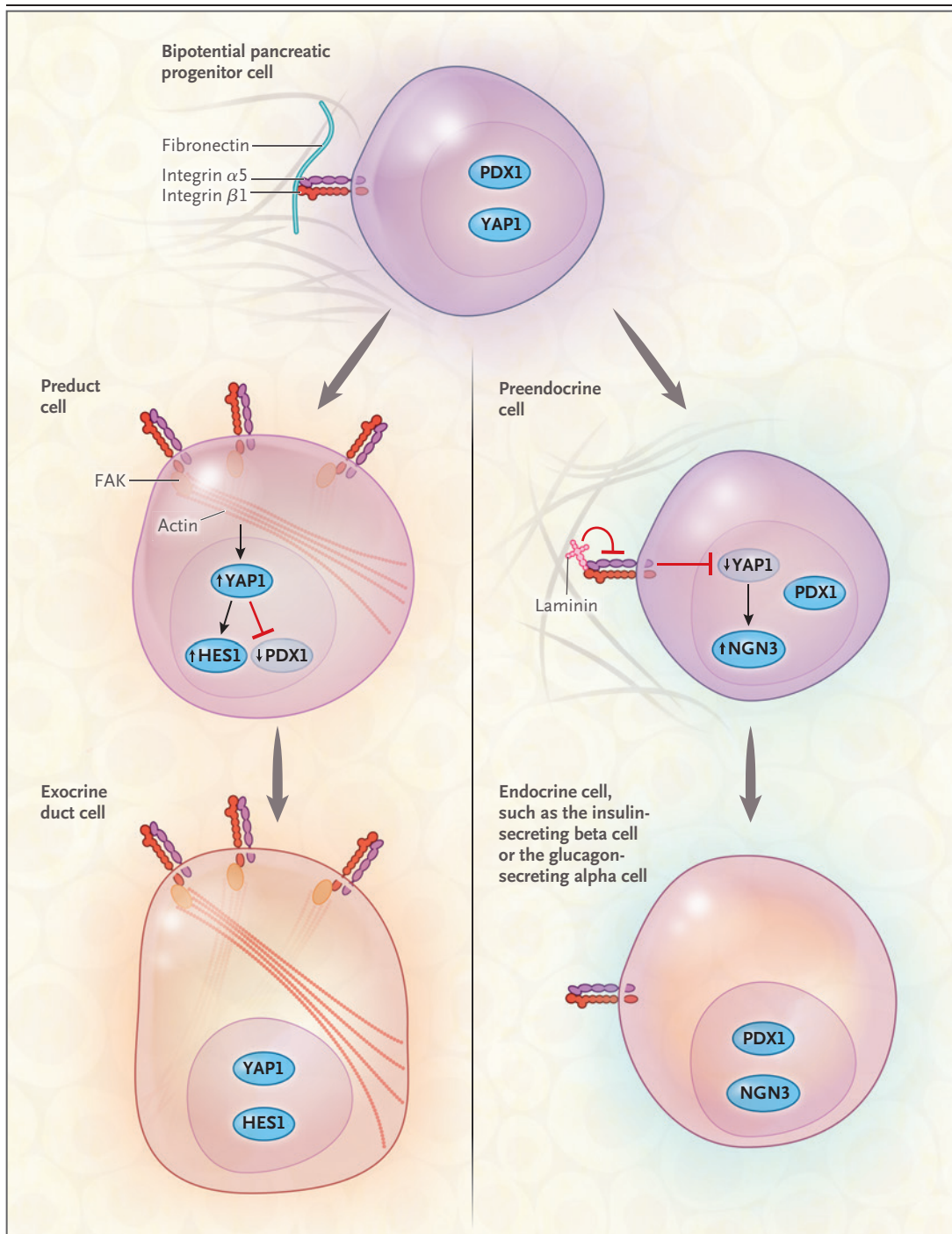
The study builds on the remarkable observation that cells at the center of cell clusters — within which the cells have been engineered to express green fluorescent protein (GFP) reporting the regulation of pancreatic and duodenal homeobox 1 (PDX1), a transcription factor — maintain PDX1 expression, whereas cells at the periphery of the clusters lose expression. Similar differentials in PDX1 expression have long been observed in the developing pancreatic buds of mice and humans: cells of the central region of the pancreas express high levels of PDX1, which is extinguished in more peripheral, differentiating acinar cells.<sup>2</sup> The core region of the pancreatic epithelium is of great interest to researchers who are aiming to recreate the ontogeny of islet endocrine cells in the laboratory and to generate replacement endocrine cells for patients with diabetes.<sup>3</sup> This part of the epithelium is the “birthplace” of insulin-producing beta cells, where bipotential protodifferentiated pancreatic

progenitors give rise to both ductal and endocrine cell types.<sup>4</sup>

Mamidi et al. sought to mimic the states of cellular confinement and peripheral expansion, similar to those observed in PDX1–GFP clusters and in the embryonic pancreas. To accomplish this, they used micropatterned plates with wells of varying sizes to either physically constrain cells or allow their spreading. The authors found that cells cultured in narrow wells retained high PDX1 expression, while cells allowed to spread in wider wells lost PDX1 expression. This effect was also observed for genes encoding other factors that are known to regulate endocrine development, such as NK6 homeobox 1 (NKX6-1) and neurogenin 3 (NGN3), but not for “nonendocrine” genes.

A different result was observed for the mechanoresponsive transcription factor yes-associated protein 1 (YAP1). Cell confinement resulted in the loss of YAP1. This loss also happens in the pancreas as bipotential pancreatic progenitors differentiate into endocrine progenitors. Indeed, differentiating endocrine cells express no appreciable YAP1. Mamidi et al.<sup>1</sup> observed increased differentiation of bipotential progenitors into endocrine cells on genetic or pharmacologic inhibition of YAP1, which suggests that YAP1 normally maintains bipotentiality and suppresses differentiation of the progenitors into endocrine cells. Activated YAP1 expressed within the epithelium, on the other hand, suppressed endocrine gene expression and promoted expression of the Notch1 target HES1 and differentiation into ductal cells. Together, these findings identify a dual role for YAP1, as both a suppressor of endocrine differentiation and an activator of ductal differentiation.

The question then arises as to what events might regulate YAP1 suppression and endocrine



**Figure 1. Mechanosignaling and Progenitor Cell Fate in the Developing Pancreas.**

A study described by Mamidi et al.<sup>1</sup> delineated how events that influence the tethering of the preendocrine cell — specifically, transient laminin signaling through integrin  $\alpha 5$  — suppress the transcription factor yes-associated protein 1 (YAP1). YAP1, in turn, normally suppresses neurogenin 3 (NGN3), which is required for differentiation of progenitor cells into endocrine cells. High levels of YAP1 signaling in ductal progenitors promote the expression of HES1, a transcription factor that, when expressed, promotes differentiation into ductal cells. The authors also showed that “stress” fibers made up of a specific filamentous form of the cytoskeletal molecule actin, as well as focal adhesion kinase (FAK), are required for maintaining high levels of nuclear YAP1.

differentiation during normal pancreatic development. The authors surmised that a cue, such as one arising in the changing milieu of the extracellular matrix that surrounds the branching pancreatic bud, may provide the fate-altering trigger. The authors found that focal adhesion kinase (FAK) signaling is required for YAP1 activity and that a reduction in FAK signaling promotes endocrine specialization (Fig. 1, left). They also found that within the heart of the developing pancreatic bud, levels of the extracellular protein laminin 1 increased dramatically before the explosion of endocrine differentiation that occurred during late embryogenesis, known as the secondary transition (Fig. 1, right). In addition, they showed that not only did integrin  $\alpha 5$  (a molecule that spans the cell membrane and adheres to the extracellular-matrix protein fibronectin, tethering the cell to the extracellular matrix) specifically decline in isolated cells that were differentiating into endocrine cells, but that plating cells on laminin could induce this decline. They suggest that disrupting fibronectin-to-integrin signaling, through laminin suppression of integrin  $\alpha 5$ , is required to induce the biomechanical forces that suppress YAP1 during endocrine differentiation. They also show that this effect is mediated through the cytoskeleton. Why, though, do only certain cells within the pool of bipotential progenitors respond to laminin 1? This might be the result of differential responsiveness due to lateral inhibition events that can occur in epithelial systems.

Mamidi and colleagues have delineated a cascade of molecular events and biomechanical forces that occur within cells as the surrounding extracellular-matrix microenvironment evolves, together acting as a spatiotemporal gatekeeper of pancreatic progenitor cell fate. They have built a compelling model in which promotion of differentiation into endocrine cells is triggered by extracellular cues that suppress YAP1, which normally suppresses NGN3. As researchers continue to refine their understanding of how functional beta cells differentiate in vivo, they are at the same time creating a road map that I believe will be used to eventually recapitulate, either in vitro or in vivo, the molecular events that direct progenitors to differentiate into islet endocrine cells.

Disclosure forms provided by the author are available with the full text of this article at [NEJM.org](http://NEJM.org).

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DOI: 10.1056/NEJMcibr1900052

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