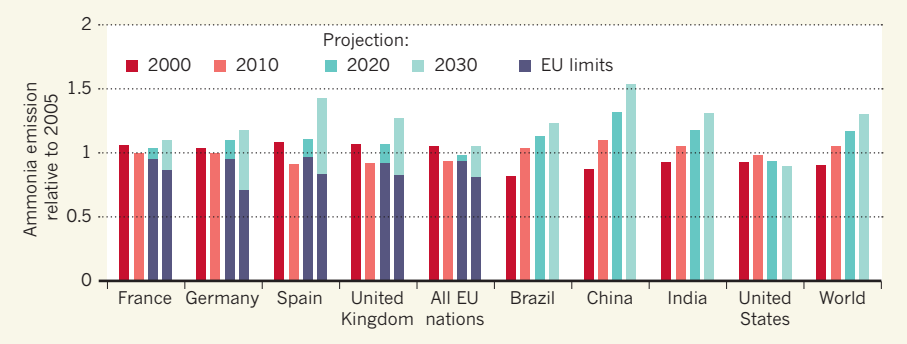

Figure 2 | Past and projected ammonia emissions. Ammonia emissions from many countries are on course to increase in the next couple of decades — including in the European Union, where several countries are set to exceed legally binding limits for 2020 and 2030. Data are shown relative to ammonia emissions in 2005 for each country. Graphs for the European countries are plotted from official EU data (see go.nature.com/2awg8sc), whereas non-EU data are from the EDGAR 4.3 inventory[11]. Projected future values are extrapolated from the most recent five years of available data. Both data sources are 'bottom-up' inventories, which combine data on the intensity of ammonia-emitting activities with estimated quantities called emission factors. Van Damme *et al.*[2] show that satellites now have the capability to help assess compliance with legally binding limits.

be made to warm-temperate and tropical climates. There is also huge potential to improve on Van Damme and colleagues' inversion technique, which assumes that the atmospheric lifetime of ammonia is constant everywhere. This simplification will underestimate ammonia emissions at sources in windy locations, such as on coasts or in mountain areas. Together, these limitations might explain why the authors do not detect high ammonia levels at any seabird colonies, which are known to be substantial ammonia hotspots, especially in sub-polar regions[10]. Curiously enough, the authors identify several fire-based sources (including the second-largest global ammonia hotspot, found in West Africa), but exclude many of these from their detailed analysis.

Perhaps the most important feature of the new analysis, however, is that it has demonstrated ammonia trends at specific locations. For example, the authors detected changes of 15–20% in ammonia levels at hotspots over the period of the study (see Fig. 4 of the paper[2]). The achievment of this accuracy for hotspots implies that even better precision could probably be achieved for observations at national and regional scales.

Achieving this capability now is especially timely. Ammonia emissions in many countries are currently increasing (Fig. 2), even in the European Union, which has committed to achieving an overall reduction of 6% by 2020 and 19% by 2030, compared with 2005 levels (see Annex II at go.nature.com/2e2gphe). Combined with atmospheric models (which are necessary for considering the effects of ammonia's interactions with acidic gases and particulate matter), satellite technology offers a valuable independent tool with which to check whether countries are really achieving their goals. ■

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DEVELOPMENTAL BIOLOGY

Location matters for insulin-producing cells

Intrinsic and extrinsic cues drive dynamic processes that control cell fate during organ development. A study of mouse and human cells reveals how these inputs affect cells that make the essential hormone insulin. SEE LETTER P.114

FRANCESCA M. SPAGNOLI

During development, cells proliferate and differentiate to enable organs to achieve their final functional architecture[1]. As cells develop to reach their mature state, they respond to various extrinsic cues provided by the surrounding microenvironment and acquire a fate that can be determined by their location in a tissue. But little is known about how these cues drive intracellular changes, such as transcription or differentiation, or how tissue architecture and cellular rearrangements can, in turn, affect cell fate. On page 114, Mamidi *et al.*[2] provide insight into how cell location and exposure to certain external cues can affect whether cells in the developing pancreas give rise to β-cells that make the protein insulin. Deficiencies in insulin-producing cells can lead to diabetes, so a better understanding of how these cells form could have clinical implications.

As well as the hormone insulin, which has an essential role in the regulation of blood-glucose levels[3], the pancreas produces digestive enzymes. The organ develops by a complex stepwise process that gives rise to many cell types[3,4]. Embryonic pancreatic cells, also termed progenitor cells, initially express the transcription factor PDX1 and can generate all of the cell types found in the pancreas[3,5]. When the pancreas begins to form, these progenitor cells are segregated into domains that give rise to specific cell lineages[5].

One domain forms the cells that make digestive enzymes, and the other domain, termed the trunk domain, develops from cells called bipotent pancreatic progenitors (bi-PPs) that can give rise to two cell types (Fig. 1), pancreatic duct cells and hormone-producing cells[4,5]. A hallmark of the bi-PP cells that will differentiate into hormone-producing cells[3,4], such as β-cells, is the expression of the transcription factor NGN3.

Mamidi and colleagues investigated what determines the type of cell that develops from a bi-PP cell. They used experimental systems that included *in vivo* mouse models and *in vitro* studies of organ cultures and human embryonic stem cells that had differentiated to form pancreatic cells.

In some of the *in vitro* studies, the authors used micropatterned glass slides to restrict the location and shape of the regions to which stem cells could attach and, hence, grow on. This revealed that cells confined to a limited space were more likely to differentiate into hormone-producing cells, and that cells that could spread out over a large area were less likely to form this type of cell.

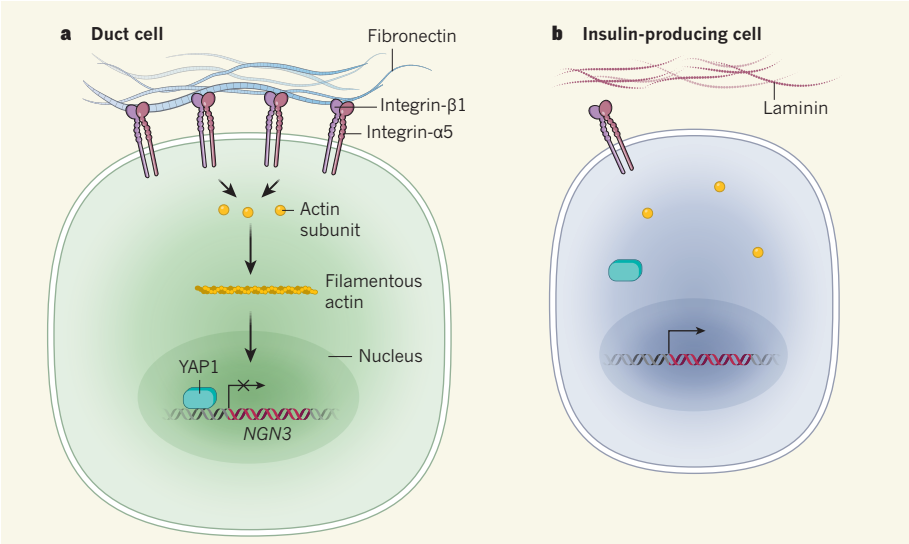

Figure 1 | Extracellular cues affect the formation of insulin-producing cells. Mamidi *et al.*[2] used mouse and human cells to investigate what determines whether a type of cell in the developing pancreas — called a bipotent pancreatic progenitor — becomes a duct cell or a cell that makes the protein insulin. **a**, The authors report that duct-cell formation is associated with the presence of the protein fibronectin in the cell's microenvironment. Cellular interaction with fibronectin can be mediated by the proteins integrin-α5 and integrin-β1, which form a fibronectin receptor. This interaction is associated with cellular spreading (an increase in surface area). In such cells, the protein actin is in a filamentous form and there are high levels of the protein YAP1 in the nucleus. YAP1 represses the transcription of the gene encoding the protein NGN3, which is characteristic of the progenitor cells that will give rise to insulin-producing cells. **b**, The authors found that the formation of insulin-producing cells is associated with the presence of the protein laminin in the cellular microenvironment. Compared with duct cells, insulin-producing cells have lower levels of integrin-α5 (and therefore lower levels of fibronectin receptors), higher expression of the gene encoding NGN3, and less filamentous actin, cellular spreading and nuclear YAP1.

How might differences in cellular shape affect cell fate? The authors tested whether the protein YAP1 might be involved. YAP1 is a component of the Hippo signalling cascade, which controls organ size, and can function as a sensor of cell shape and a mediator of cellular responses to mechanical stimuli[6]. In the cells cultured on a micropatterned surface, cellular spreading was associated with sustained nuclear activity of YAP1 and low levels of PDX1 and NGN3, whereas cell confinement corresponded to low levels of YAP1 in the nucleus and high levels of PDX1 and NGN3. The authors then engineered mice that lack YAP1 in their pancreatic progenitor cells. The number of insulin-expressing cells in these animals was higher than that in mice that had normal YAP1 expression, providing *in vivo* results that are consistent with their *in vitro* work.

Mamidi *et al.* sought to define the downstream targets of YAP1 in pancreatic progenitor cells. They found that YAP1 regulates the signalling pathway that is mediated by the protein Notch, and also that YAP1 directly represses transcription of the gene that encodes NGN3.

The authors also wanted to understand the pathway upstream of YAP1. How does an extracellular cue regulate YAP1, and does the same cue trigger the generation of hormone-producing cells? Changes in the arrangement of the protein actin in cells can affect YAP1 activity and cause changes in cell shape[6]. Using human embryonic stem cells that had differentiated into pancreatic cells and mouse pancreatic tissue grown *in vitro*, Mamidi *et al.* showed that blocking the assembly of actin into filaments resulted in cells having a reduced surface area, low nuclear YAP1 levels and high NGN3 levels, compared with cells in which filamentous actin assembly had not been perturbed. It remains to be determined exactly how actin regulation converges on a YAP1-mediated signalling pathway to govern cell fate.

The most interesting aspect of Mamidi and colleagues' work is undoubtedly their finding that YAP1 activity in a cell responds to external cues from the pancreatic microenvironment that affect the assembly of actin filaments. To investigate how such external cues might affect actin and YAP1, the authors focused on integrin and FAK proteins, which function at a 'crossroads' between filamentous actin and the surrounding microenvironment. These proteins can mediate the interactions between cells and the extracellular matrix material in their surroundings[6]. The authors report that a high level of integrin-α5 in mouse or human cells correlates with cells that are in a bi-PP state or cells that are duct progenitors, whereas low levels of this integrin are associated with the formation of hormone-producing cells. Integrin-α5 and integrin-β1 can form a receptor for the extracellular protein fibronectin[7]; therefore, a change in integrin expression might affect the ability of a pancreatic progenitor cell to respond to the extracellular environment in a way that affects cell fate.

The authors' studies of mouse and human cells show that the presence of extracellular fibronectin promotes cellular spreading, whereas exposure to an extracellular matrix protein called laminin inhibits spreading and is accompanied by a reduction in the level of integrin-α5 and in the level of YAP1 in the nucleus. Perhaps a feedback loop based on signalling between extracellular-matrix material and integrin receptors exists in which pancreatic progenitor cells that are exposed to laminin have low expression of integrin-α5, lose the ability to respond to fibronectin, and develop into insulin-producing cells. But how the extracellular matrix might control integrin expression is an open question.

Mamidi *et al.* suggest that regions of different extracellular-matrix composition in the developing pancreas microenvironment might exert different effects on progenitor cells, thereby influencing cell fate. The authors characterized the distribution of fibronectin and laminin in the developing mouse pancreas, and noted that the cells that will develop into insulin-producing cells are more commonly found in association with laminin than with fibronectin. However, these developing insulin-producing cells are also exposed to some fibronectin, suggesting that cells might have to respond to complex gradients of extracellular-matrix material *in vivo*.

The authors' analysis of interactions between the extracellular matrix and cells through static 'snapshots' of the process offers a valuable starting point. However, it would be even better to understand the dynamics of the interactions in time and space in an *in vivo* context, given that the deposition of extracellular-matrix material and cellular positions change constantly during development. It would also be fascinating to discover which cells deposit the extracellular material that surrounds the developing pancreas.

Mamidi and colleagues' work might have direct implications for efforts to generate β-cells for cell-replacement therapies to treat diabetes. Their study suggests avenues of investigation for improving the strategies used to coax human embryonic stem cells to differentiate into insulin-producing cells[8]. ■

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