Insights & Perspectives

β-Cell evolution: How the pancreas borrowed from the brain

The shared toolbox of genes expressed by neural and pancreatic endocrine cells may reflect their evolutionary relationship

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Introduction

When two systems or cell types in the body are found to have similar morphologies or physiologies, this is often attributed to their common developmental origin. However, if a common developmental origin cannot be found, then a shared evolutionary ancestor may be hypothesised to explain why they are alike. Such is the case with cells of the nervous system and pancreatic β-cells located in the islets of Langerhans in mammals. Although these cells have numerous physiological, functional and developmental similarities, they are not derived from a common tissue or even the same embryonic germ layer. We will argue that β-cells evolved by co-opting a neuronal transcription program (a set of neuronal transcription factors under the control of a master regulator). This understanding will offer insight into their function and development and provide clues into how pancreatic cells, particularly β-cells, can be derived from other sources, such as embryonic stem cells. Proper β-cell function may depend on turning on this neuronal program,

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and methods to do so could be borrowed from the successful derivation of neurons from neural stem cells and embryonic stem cells.

β-Cells do a great neuron impression

The resemblance between neurons and pancreatic β-cells ranges from their physiology and function, to gene expression and pathways utilised in their development. These similarities are described in Table 1. The physiological similarities between endocrine cells and neurons were first recognised and summarised in the amino precursor uptake and decarboxylation (APUD) [1] and paraneuron concepts [2]. These formed the basis for suggestions that the endocrine and nervous systems have a common evolutionary origin [3]. Basically, the way that β-cells store insulin, receive and process external stimuli and in response release insulin almost exactly mimics the way neurons store and release neurotransmitters even down to the microvesicle assembly

machinery used [4–8]. Neurons and β-cells also share organisational features such as cell adhesion molecules, migration signals and association with support cells [9–11].

It is, therefore, not surprising that β-cells and neurons have similar gene expression patterns [12–18]. One interesting example of this is the fact that both β-cells and neurons lack expression of repressor element 1 silencing transcription factor (REST) which is expressed in non-neuronal cells and suppresses the neuronal phenotype [14]. Target genes of REST, such as the exocytosis protein synaptotagmin, have been implicated in the expression and release of insulin [14]. Also, REST has been shown to regulate Pax-4, a transcription factor crucial for the maturation of β-cells [19].

As one would expect considering these gene expression similarities, there are many developmental pathways shared between β-cells and neurons [20–24]. In particular, the interaction between notch and neurogenin plays a role in both neuronal and endocrine cell fate specification [20, 21]. It is also intriguing that many genes that were thought to be pancreas-specific, such as PDX1, HB9 and Islet-1, are transiently expressed during neural development [23, 24].

In addition to their in vivo similarities, there are also in vitro parallels. It has been shown that adult pancreas-derived precursors can be grown and

Table 1. Similarities between β-cells and neurons

Category	Similarity	Description
Physiology and function	APUD phenotype & paraneuron concept	All endocrine cells have the ability to take up and decarboxylate amine precursors, as well as produce polypeptide hormones [1], features that they share with neurons [2].
	Neurotransmitters	β-Cells synthesise glutamate and use it for intracellular signalling in glucose-responsive insulin secretion [4].
	Neurotransmitter assembly proteins	β-Cells express glutamic acid decarboxylase, an enzyme found in gamma aminobutyric acid (GABA)-secreting neurons but not other cell types [5].
	Neurotransmitter receptors	β-Cells contain glutamate receptors, which are mainly found in the central nervous system [6].
	Secretory granules and microvesicles	β-Cells store insulin in secretory granules that are secreted from synaptic-like microvesicles [5].
	Action potentials	Pancreatic β-cells are capable of generating action potentials similar to those used by neurons to transmit signals along their axons. These action potentials may cause the release of insulin from β-cells in a manner akin to the release of neurotransmitters from neurons [7].
	Glucose response	Neurons in the hypothalamus can sense blood glucose levels and are stimulated by changes in the same way β-cells are [8].
	Schwann cells	Islets are surrounded and highly penetrated by Schwann cells, the major glial cell of the peripheral nervous system [9]. These Schwann cells may be functioning as support cells for both the islets and innervating neurons [9].
	Cell migration	The migration of pancreatic precursors into the surrounding mesenchyme has been shown to be dependent on the axon guidance protein, netrin-1 [10].
	Adhesion molecules	Endocrine cells of adult mammalian islets associate partially by the expression of neural cell adhesion molecule (NCAM) [11].
Gene expression	Global gene expression	β-Cells are more similar in global mRNA expression and chromatin methylation pattern to neurons than any other cell type, including pancreatic acinar cells [12].
	Sodium channels	Islet cells express the alpha-1 subunit sodium channel mRNA which is primarily expressed in the brain [13].
	Neurofilaments	Dissociated β-cells have been found to synthesise neurofilaments in vitro which may be recapitulating their developmental migration [5].
	REST expression	β-Cells lack expression of repressor element 1 silencing transcription factor (REST) which is expressed in non-neuronal cells and suppresses the neuronal phenotype [14].
	Insulin and other pancreatic endocrine hormones	Insulin, glucagon and ghrelin are expressed in the brain during development and in adulthood [15, 16].
	Glucose transporters	The β-cell specific glucose transporter, Glut-2, is expressed in certain regions of the brain, including the hypothalamus, one of the sites of insulin action [17].
	Isl-1	The homeodomain protein Isl-1 is expressed in mature pancreatic endocrine cells, calcitonin-producing thyroid cells and neurons of the peripheral and central nervous systems [18].
Development	Pax-6	Pax-6 is involved in development of α-cells of the pancreas and proper insulin secretion from β-cells [20], as well as neurogenesis in the developing central nervous system [21].
	Nkx6.1	Nkx6.1 is a transcription factor involved in the formation of β-cells in the pancreas [20] as well as maturation and migration of hindbrain motor neurons [22].
	Notch	Notch is a transmembrane signalling protein that has been implicated in maintaining pancreatic precursors in a proliferative state, as well as influencing cell fate decisions [20]. Notch has been shown to have similar functions in the developing nervous system [21].
	Neurogenin	The transcription factor neurogenin-3 is repressed by Notch and when activated it contributes to specification of endocrine cells in the pancreas [20]. Notch may also repress neurogenin-1 and 2 which are involved in the specification of neurons from neural progenitors [21].
	HB9	HB9 is expressed in the embryonic gut and initiates formation of the pancreatic bud and is later expressed in mature β-cells[20]. BHB9 is also expressed in embryonic and adult motor neurons [23].
	PDX1	The pancreatic specific transcription factor PDX1 is turned on in the brain during development [24].

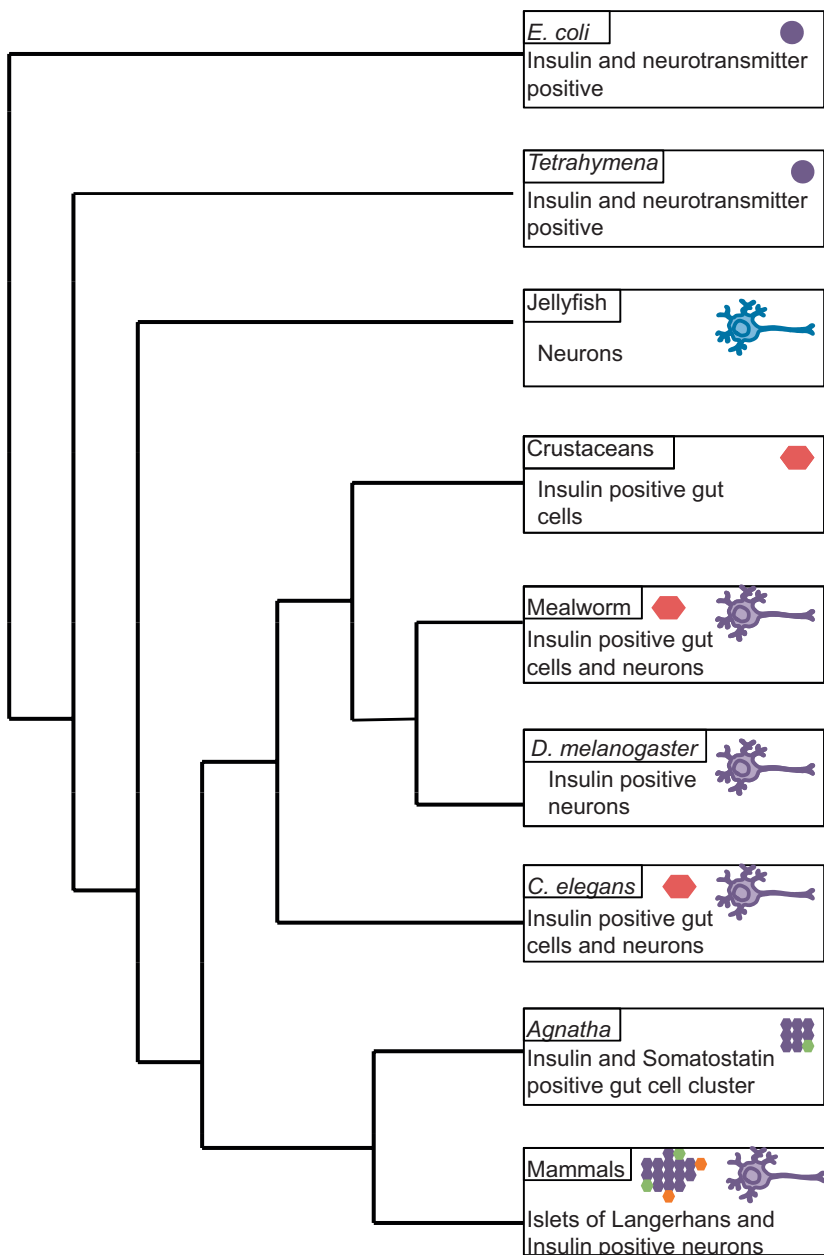Ideas & Speculations

Ideas & Speculations

Figure 1. Schematic of a phylogenetic tree depicting known insulin expression in various organisms. Insulin and neurotransmitters are seen to be co-expressed in multiple cell types and through multiple kingdoms suggesting that they share regulatory transcriptional elements and that these elements are highly conserved. Red = insulin expression, blue = neurotransmitter expression, purple = insulin and neurotransmitter expression, circle = single celled organism, hexagon = gut cell.

differentiated in culture in a manner similar to neural stem cells and that they are capable of making pancreatic cells as well as neural cells, especially neurons [25]. Recently it has been found that insulin-expressing cells in the pancreas are also able to form neurons in vivo [26]. This suggests that pancreatic β-cells are so similar to neurons that the conversion from the former to the latter can happen without any external manipulation.

Developmental origin of the pancreatic β-cells

One possible explanation for the similarities between β-cells and neurons is that they have a common develop-mental origin. In fact when the APUD series was first proposed, it was hypothesised that all hormone-producing cells, including pancreatic β-cells, were derived from a common ancestor tissue – the neural crest [1]. This was based on experiments looking through embryonic development at the positional association between APUD cells and cells from the neural crest. Although many APUD cells have since been verified to be of neural crest origin, this is not the case with pancreatic endocrine cells. When chick embryos are grafted with the neural crest from quail and are allowed to develop, the quail neural crest cells migrate to predicted locations and contribute to the enteric nervous system but they are not involved in forming pancreatic endocrine cells [27]. In addition, when the neural tube is removed from rat embryos prior to neural crest migration and the embryos are allowed to continue developing, the pancreatic exocrine and endocrine cells are still capable of forming [28]. This indicates that pancreatic endocrine cells do not arise from the ectoderm-derived neural crest.

Like other organs of the digestive system, the pancreas is formed from the definitive endoderm [20]. Development of the pancreas begins with the expression of pancreatic and duodenal homeobox 1 (PDX1) in the primitive foregut [20]. These cells give rise to the dorsal and ventral pancreatic buds which proliferate into the surrounding mesenchyme to form both the exocrine and endocrine tissues of the pancreas [20]. Interestingly, PDX1-expressing cells of the foregut also form part of the duodenum, further evidence of the endodermal origin of the pancreas [20]. So, although the neural crest does innervate the pancreas in the form of the peripheral nervous system [27], it does not contribute to the formation of pancreatic β-cells. Taken together, these data strongly indicate that a common developmental origin cannot explain the similarities between β-cells and neurons.

β-Cell evolution

The answer to why β-cells are so much like neurons may be found in the phylogeny of the β-cell (Fig. 1). Insulin-like

proteins have been found in single celled organisms such as the bacterium *Escherichia coli* and the protozoa *Tetrahymena*, indicating that insulin may have an evolutionarily conserved function in intercellular signalling [29]. Interestingly, it has been found that protozoa synthesise the neurotransmitters norepinephrine and serotonin, suggesting that these primitive unicellular organisms have neuronal-like properties [30]. The jellyfish (*Aurelia aurita*) does not contain insulin-expressing cells in its gut [31], but does have an extensive neural network. This suggests that neurons may have evolved before β-cells and probably already contained a master transcriptional regulator that permitted expression of neuron-specific genes. A β-like cell may have appeared in an ancestor resembling the nematode *Caenorhabditis elegans*. These animals have cells in both the gut and nervous system that produce insulin which controls development as well as cellular responses to food intake [32]. Insulin has also been detected in the hepatopancreas of crustaceans such as the lobster (*Homarus gammarus*) and shore crab (*Carcinus maenas*) [31]. The fruit fly *Drosophila melanogaster* seems to be an exception as this animal does not have insulin-expressing cells in its gut but instead relies on insulin-expressing neurons in its brain to control circulating glucose levels [33]. Presumably in this fly lineage, the insulin-expressing cells were lost from the gut. In other insect species, such as the mealworm (*Tenebrio molitor*), insulin-like proteins have been found in both the gut and brain [34]. The ability of neurons to assume the function of gut β-cells is further evidence for their functional similarities. The first evidence of a conglomeration of insulin-expressing cells in an islet-like structure is found in the jawless fish *Agnatha* [35]. These islets represent the beginnings of the vertebrate endocrine pancreas as they measure blood glucose rather than gut glucose and also contain a small fraction of somatostatin-expressing cells [35]. In summary, neurons are seen in the most primitive organisms, in some cases expressing insulin to control blood sugar levels, while insulin-expressing gut cells do not appear until later in evolutionary history. This suggests that their common tool box of genes evolved

in neurons first and was then co-opted by β-cells and not vice versa.

How did β-cells acquire a neuronal program?

Most likely, the acquisition of a neuronal program in β-cells is a case of convergent evolution. It is possible that once a neuronal program had evolved (a series of genes controlled by a master transcription factor regulator), then a mutation in the promoter region of a neuronal master transcription factor, allowed it to be activated by a transcription factor expressed in certain cells of the gut (Fig. 2A). The neuronal program would then be expressed in these gut cells. This leads us to wonder whether this initial endocrine cell was a common endocrine cell that expressed many hormones (Fig. 2B) or a β-like cell that only expressed insulin (Fig. 2C), and arguments could be made either way. The idea that this ancestral cell was a common endocrine cell is supported by the fact that all endocrine cells in the pancreas and gut have some neuron-like properties [1, 2] and that the developing brain expresses hormones other than insulin, such as glucagon and ghrelin [15]. In addition, during pancreatic development, endocrine cells do not come from insulin-positive cells meaning they come from a general endocrine precursor and not a β-cell like precursor [36]. However, it is not necessary that development recapitulates the evolutionary process and there is also evidence that the initial gut/pancreas endocrine cell was a β-cell. For example, many genes that are expressed early in pancreatic development, such as PDX1 and HB9, are later expressed exclusively in β-cells [20]. Also, progenitors in the adult pancreas express insulin and are capable of giving rise to other endocrine cells [26]. It is also unclear whether other endocrine cells display all the neuronal properties that β-cells do. This debate could be resolved by knowing whether organisms with insulin-expressing cells in their gut, such as the molluscs and crustaceans, also have other endocrine cell types (α-cells, for example). Also, do the insulin-expressing cells in these organisms have the same neuronal-like properties as the β-cells described in vertebrates?

Another interesting question that arises from this hypothesis is what is the neuronal master transcription factor that made this switch? A good case can be made for neurogenin (Ngn) being this master regulator, although other candidates include NeuroD and Islet-1. Ngn-1 and -2, and Ngn-3 are expressed in precursors in the developing brain and pancreas, respectively [20, 21]. In these tissues Ngn plays a critical role in specifying precursor fate to the neuronal and endocrine lineages in favour of other cell types (glial and exocrine cells) by inducing expression of other transcription factors, signalling factors and cytoskeletal proteins [20, 21]. Additionally, overexpression of Ngn-3 in combination with PDX1 and MafA in acinar cells is sufficient to convert these cells to β-cells, further evidence of its role in endocrine fate determination [37]. Knowing which transcription factor was responsible for the evolution of β-cells with neuronal characteristics is important to studying β-cell function and differentiation from precursor or stem cells.

Conclusions

β-Cells and neurons share many similarities in their physiology, function, development and gene expression. This can be explained by cells of the gut co-opting a neuronal transcriptional program leading to the evolution of β-cells. There are many questions to be answered in order to clarify just how this happened, including when insulin-expressing cells in the gut acquired a neural identity and whether the ancestral endocrine cell was a β-cell or a common endocrine precursor.

One area where this may have significant implications is stem cell biology. Diabetes is a disease of growing concern and many efforts are being made to regenerate β-cells from both adult tissue and embryonic stem cells. However, there are still no protocols that result in the differentiation of a large quantity of purified, functionally mature β-cells. Methods used for deriving functional neurons from stem cells could be applied to β-cells, especially given that adult pancreatic precursor cells can be cultured in conditions identical to those used for adult neural stem
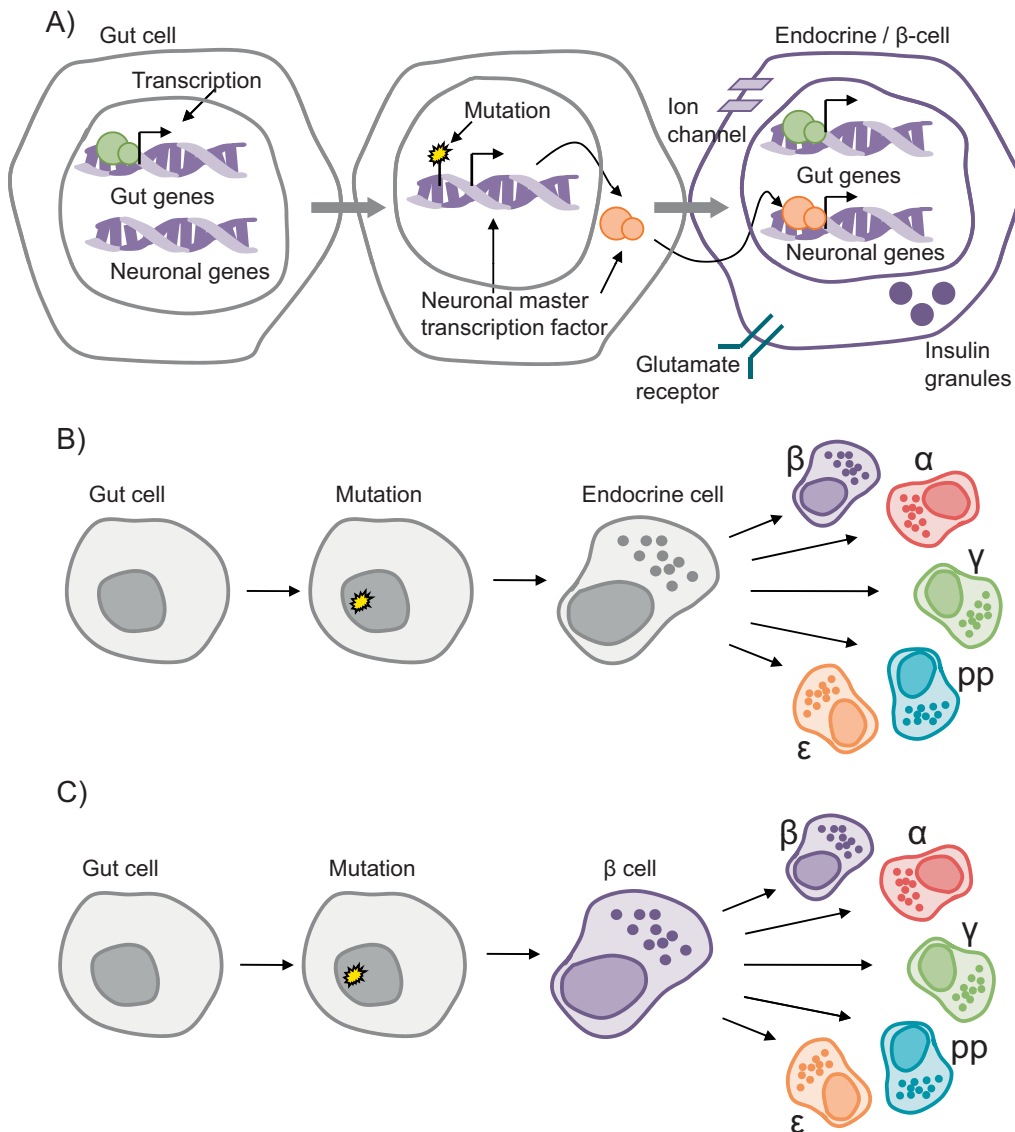
Figure 2. A: Convergent evolution: A mutation in a master transcription regulator of the neuronal program allowed it to be activated in a gut cell resulting in expression of neuronal genes in certain gut cells. **B:** The first endocrine cell in the gut was a common endocrine cell that expressed and secreted multiple hormones. Specialised hormone-producing cells found in the mammalian pancreas evolved from this endocrine cell. **C:** The first endocrine cell in the gut was an insulin-expressing β-like cell. Through evolution this β-cell gave rise to the other endocrine cells of the pancreas.

cells [25]. Also, if turning on a neural program is required for proper β-cell function then this would be an important feature to look for in stem cell-derived β-cells.

There are some differences between β-cells and neurons that would need to be considered if using these techniques. Besides the obvious differences in their in vivo function and structure, there are differences in their glucose-sensing and response mechanisms. For example, glucose-sensing neurons are capable of metabolising and responding to glycerol and galactose (which β-cells cannot) [8]. Also, neurons do not express a critical β-cell subunit of ATP-dependent potassium channels [8]. These and other key differences will need to be understood as they may significantly impact the function of a stem cell-derived β-cell.

The take-home message here is the importance of considering biological properties in the context of evolution. It is not enough to know that certain cell types express certain genes and behave in a certain fashion, we also need to understand why this is so. The case of the pancreatic β-cell behaving and developing like a neuron is one example of how an understanding of the evolution of this process may help us understand the system as a whole.

Acknowledgments

Ideas & Speculations

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