Differential use of the Nkx2.2 NK2 domain in developing pancreatic islets and neurons

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The homeodomain transcription factor (TF) Nkx2.2 governs crucial cell fate decisions in several developing organs, including the central nervous system (CNS), pancreas, and intestine. How Nkx2.2 regulates unique targets in these different systems to impact their individual transcriptional programs remains unclear. In this issue of *Genes & Development* Abarinov and colleagues (pp. 490–504) generated and analyzed mice in which the Nkx2.2 SD is mutated and found that the SD is required for normal pancreatic islet differentiation but dispensable for most aspects of neuronal differentiation.

In the pancreas, Nkx2.2 is initially expressed in acinar-, duct-, and endocrine-competent multipotent progenitors before becoming restricted to endocrine precursors and then three of the six endocrine islet cell types: insulin$^+$ β cells, a subset of glucagon$^+$ α cells, and PP cells (Sussel et al. 1998; Prado et al. 2004). Genetic loss of *Nkx2.2* induces an islet cell fate switch: While β cells are entirely lost and α cells are reduced, showing Nkx2.2 to be required for their specification, the ghrelin$^+$ ε cell population is concomitantly expanded (Sussel et al. 1998; Prado et al. 2004). Postnatally, Nkx2.2 expression in mature β cells is required for their function and for maintaining the β cell state (Gutiérrez et al. 2017). Similarly, in the early developing spinal cord, multipotent ventral p3 neuronal progenitors express Nkx2.2, and its loss in *Nkx2.2*-null mice manifests in a ventral to dorsal cell fate switch (Briscoe et al. 1999). Motor neurons are expanded due to removal of Nkx2.2-mediated repression of the motor neuron determinant Olig2 (Briscoe et al. 1999). This comes at the cost of a loss of ventral V3 interneurons and serotonergic neurons, while oligodendrocyte maturation is delayed (Briscoe et al. 1999).

The structurally conserved regions of Nkx2.2 comprise the DNA-binding homeodomain, tinman (TN) domain, and NK2-specific domain (SD) that defines NK-2 family TFs in both mammals and invertebrates. The TN domain is required for efficient Nkx2.2 interaction with Groucho corepressors and is necessary for correct cell fate specification in both the pancreas and spinal cord via transcriptional repression. The highly conserved (95%) nature of the SD points to a potentially important role in Nkx2.2 function (Watada et al. 2000) but has received scant attention until now. Most analyses of SD function have been performed in vitro and yielded contrasting conclusions specific to the cellular paradigm used (Watada et al. 2000; Zhang et al. 2020).

Now, Abarinov et al. (2023) elegantly show that while the SD is required for proper cell fate allocation in the pancreas, it is apparently dispensable in the CNS. Pancreatic β cells are dramatically depleted in SD mutant animals owing to the arrested differentiation of β cell precursors, culminating in neonatal diabetes and perinatal lethality. Concurrently, ghrelin$^+$ and somatostatin$^+$ δ cells and, later, glucagon$^+$ cells are expanded (Abarinov et al. 2023). Thus, the Nkx2.2 SD is required in the β cell lineage both for ensuring their proper differentiation and to repress alternative endocrine non-β cell fates during later pancreas development. While the SD mutant phenotype largely mirrors that of *Nkx2.2*-null mice, δ cell expansion is unique to the SD mutant. This might be due to the SD only being required for secondary-phase β cell differentiation closer to the later stages of pancreas development, when δ cells arise (the glucagon$^+$ and ghrelin$^+$ cell expansion seen in E12.5 *Nkx2.2*-null embryos does not occur in E12.5 SD mutants). This is consistent with β cell loss not being fully compensated for via expansion of non-β endocrine cell types in the SD mutant. The late requirement for SD function means that the temporal window for compensatory endocrine differentiation is more limited with endocrine neogenesis nearing completion.

Furthermore, β cell-specific SD mutation impaired β cell function, associated with defective glucose-sensing and insulin secretion, and in addition derepressed neural markers, showing the Nkx2.2 SD to be required in β cells for their maturation and function and silencing of neuronal transcripts incompatible with this (Abarinov et al. 2023). As Nkx2.2 is expressed in α cells and PP cells

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alongside β cells in the postnatal islet (Sussel et al. 1998), it would be interesting to determine whether the SD is similarly required for their mature function.

In contrast, Abarinov et al. (2023) failed to uncover a significant phenotype in the CNS of SD mutant mice prior to birth with no significant changes detected in the differentiation of V3 interneurons, serotonergic neurons, or oligodendrocytes. Likewise, SD mutation failed to perturb Nkx2.2-mediated suppression of Olig2-driven motor neuron differentiation both in vivo and in vitro. Thus, the SD appears to be dispensable both for generation of neurons dependent on Nkx2.2 and for its repression of motor neuron fate allocation. It is not inconceivable, however, that compromised functionality of neurons derived from SD mutant CNS progenitors might only emerge after birth, detection of which is precluded by the perinatal lethality of SD mutants. Generation and analysis of CNS-specific SD mutants will be required to test this, as acknowledged in the present study.

It will be interesting to determine whether the SD is required to confer Nkx2.2 function in other tissues where it is expressed besides the pancreas, such as the developing intestine, where it is expressed in a subset of enteroendocrine cells (Desai et al. 2008). As in the pancreas and CNS, Nkx2.2 is required for proper cell fate allocation: Nkx2.2 deletion results in deficiency of several enteroendocrine cell populations in the duodenum and jejunum, while ghrelin$^+$ cells are expanded, as in pancreatic islets (Desai et al. 2008). Given this conserved function of Nkx2.2, including acting upstream of the TF Pax6 in both the pancreas and intestine, it is tempting to speculate that the SD will be similarly required for proper intestinal enteroendocrine cell fate allocation.

It has been suggested that in the *Drosophila* homolog Vnd, the SD functions to stabilize its interaction with the Groucho corepressor, but the effect of SD mutation on repressive activity is moderate (Uhler et al. 2007). As Nkx2.2 SD mutant mice fail to phenocopy mutants for the Groucho-binding TN domain and since SD and TN domain mutations perturb interactions with distinct sets of proteins (Papizan et al. 2011; Zhang et al. 2020), it appears unlikely that the SD functions solely to promote TN-Groucho-mediated repression in Nkx2.2. In contrast to *Drosophila* work, in vitro studies dissecting Nkx2.2 domain function concur that the SD likely suppresses the function of the adjacent C-terminal domain but deviate in whether this C-terminal domain acts as a transcriptional activator or repressor, likely due to differences in cellular context between the in vitro models used (Watada et al. 2000; Zhang et al. 2020). This C-terminal masking function is likely also conserved in the related NK-2 TFs Nkx2-1 and Nkx2-5 (Watada et al. 2000), raising the possibility that similar to Nkx2.2, the SD governs tissue-specific functions in these and other NK2 TF family members. The hydrophobic core of the SD has been proposed to resemble motifs that act as interfaces for protein–protein interactions in other TFs (Apergis et al. 1998). Consistent with this, through mass spectrometry of MIN6 cells transfected with Nkx2.2 domain mutants, Abarinov et al. (2023) show that SD mutation perturbed interactions with members of the cohesin complex, various chromatin modifiers, and components of the nuclear pore complex (NPC). As this analysis failed to show any gain of interactions between the SD mutant and coactivators, it is incongruent with the SD attenuating a C-terminal domain with an activator function. Especially in light of conflicting reports on its transcriptional nature, and to exclude the possibility that the Nkx2.2 C-terminal domain functions as a transcriptional repressor, it might prove insightful to examine the effects of C-terminal mutation in a pancreatic context. As for the current study, interpretation of the results will be hindered by Nkx2.2 functioning as both a transcriptional repressor and activator in the pancreas. Regarding the SD potentially interacting with the NPC, and consistent with the finding by Abarinov et al. (2023) of both up-regulated and down-regulated SD-dependent transcriptional targets, NPC proteins are capable of mediating both activating and repressing downstream processes (Pascual-Garcia and Capelson 2021). Furthermore, the SD was found to mediate interactions between Nkx2.2 and many cofactors participating in these processes (Abarinov et al. 2023). Abarinov et al. (2023) propose that the SD might regulate both activation and repression by guiding Nkx2.2-bound targets to the NPC and/or by promoting interaction between such targets and chromatin modifiers within the NPC. Further work will be required to test these hypotheses.

Notwithstanding the possibility of postnatal neuronal defects in CNS-specific SD mutants, Abarinov et al. (2023) uncovered a novel role for the SD in divorcing the function of Nkx2.2 in the pancreas and CNS, conferring pancreas-specific roles. This study provides valuable insight into TF-intrinsic mechanisms conferring their cell- and tissue-specific functions.

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