VIP Pipes Up: Neuronal Signals Direct Tubulogenesis

Jessica Houtz1 and Rejji Kuruvilla1,*
1Department of Biology, Johns Hopkins University, 3400 North Charles Street, 224 Mudd Hall, Baltimore, MD 21218, USA
*Correspondence: rkuruvilla@jhu.edu
http://dx.doi.org/10.1016/j.devcel.2014.08.011

Biological tubes serve as the body’s plumbing system, transporting fluids and gases throughout secretory, circulatory, and respiratory organs. In this issue of Developmental Cell, Nedvetsky et al. (2014) find that vasoactive intestinal peptide (VIP), secreted by parasympathetic nerves, is a surprising player in directing epithelial tubulogenesis in salivary glands.

All peripheral organs and tissues are innervated by the autonomic nervous system, which sends signals to regulate organ homeostasis. In adult organs, the sympathetic and parasympathetic branches of the autonomic nervous system control diverse physiological processes, including circulation, respiration, body temperature, digestion, and metabolism. The onset of peripheral innervation is coincident with embryonic stages of growth, differentiation, and maturation of innervated organs, yet little is known about the functional contribution of innervation to organogenesis. The emerging view that dysfunction of neuronal elements in peripheral organs may instigate pathogenesis of disease, including diabetes and heart failure (Hasan, 2013; Saravia and Homo-Delarche, 2003), underscores the need to understand the relevance of these contributions. In this issue of Developmental Cell, Nedvetsky et al. (2014) provide new insight into the role of innervation in organogenesis.

Tubes are the basic building blocks of a number of organs including the lungs, vascular system, kidneys and exocrine glands. They are the biological pipes that carry vital gases, nutrients, hormones, and waste. Epithelial tubes are established by diverse cellular mechanisms that include budding, wrapping, folding, and invagination of polarized sheets of cells to create hollow lumens (Andrew and Ewald, 2010). After tube formation, lumen size is controlled by changes in cell number, shape, and physical forces of luminal pressure and flow. Epithelial tubes are also surrounded by a stromal compartment that includes mesenchymal cells, endothelial cells, and nerves. However, the role of the neighboring vasculature and nerves in the establishment of functional interconnected tubular networks has received little attention. Nedvetsky et al. (2014) provide evidence that parasympathetic nerves instruct tubulogenesis in embryonic mouse submandibular glands (SMG) and define the neuropeptide vasoactive intestinal peptide (VIP) as a master regulator of lumen formation and expansion.

Explants of embryonic mouse SMG provide a powerful and physiologically relevant model system to study organogenesis in culture while preserving intimate contacts with blood vessels and nerves (Patel et al., 2006). In vivo, the SMG appears as rudimentary epithelial buds at midgestation that then undergo branching to form stalks and terminal buds, generating microlumens that are later joined into a continuous ductal passage. Finally, lumenal expansion allows for passage of liquids through the mature duct. SMG morphogenesis occurs in tandem with the appearance of nerves that originate locally from parasympathetic ganglia and are directed to terminal buds by the epithelium-derived neurotrophic factor, neurturin.

To examine the role of the autonomic nervous system in tubulogenesis, Nedvetsky et al. (2014) used a neurturin function-blocking antibody to deplete innervation and observed that lumen formation is disrupted in explanted SMGs. Similar results were observed in salivary glands in vivo in mice lacking peripheral nerves. The authors initially focused on the parasympathetic neurotransmitter acetylcholine as the potential nerve-derived signal regulating SMG lumen formation. In an earlier study, Knox and colleagues had found that cholinergic signaling through muscarinic (M1) receptors maintains a multipotent progenitor pool during SMG branching (Knox et al., 2010). However, in their current work, the authors observed that ductal formation is not impaired by pharmacological inhibition of muscarinic receptor signaling, despite salivary gland hypoplasia due to a drastic reduction in resident progenitor cells. Thus, the authors reasoned that other neuronal signals must instruct SMG tubulogenesis. They therefore mined an available gene expression data set generated from developing salivary glands, leading to the identification of the neuropeptide VIP as a candidate regulatory factor. VIP is a cotransmitter in the mature autonomic nervous system with well-characterized effects on immune function and release of hormones and catecholamines, although VIP’s functions during organogenesis are less clear. Nedvetsky and colleagues found that expression of the VIP receptor (VIPR) is coincident with lumen formation and is restricted to the SMG epithelium. Importantly, a VIPR peptide antagonist disrupted lumen formation, whereas exogenous VIP was sufficient to promote ductal growth in rudimentary SMG explants in the absence of nerves. VIP also functions at later stages in lumen formation, inducing coalescence of microlumens into one continuous duct and rapidly promoting an expansion of luminal cavities, as revealed by live imaging. Finally, exogenous VIP elicited dilation of existing primary duct lumens in SMG explants, implying distinct regulatory effects of VIP on lumen formation and expansion.

How does VIP control the diverse morphogenetic events of lumen initiation, fusion, and expansion? The authors...
demonstrate that cyclic AMP (cAMP)/protein kinase A (PKA) signaling acts down-
stream of VIP and is a shared regulatory mechanism in these processes. A mem-
brane-permeable cAMP analog recap-
pitulated all the effects of VIP, whereas
pharmacological inhibition of PKA activity
abrogated VIP-dependent lumen forma-
and expansion. However, the down-
stream pathways that elicit distinct
functional outcomes remain undefined.
While the authors show that VIP activates
the transcription factor CREB in salivary
epithelia, likely promoting the mitogenic
events needed for lumen growth, VIP-
stimulated luminal expansion occurs on
a timescale of 10 min, seemingly pre-
cluding transcriptional regulation. Addi-
tionally, although apoptosis has been
considered a primary mechanism under-
lying the creation of a hollow lumen (And-
rew and Ewald, 2010), the authors
observed no evidence of cell death in
VIP-treated ducts, and tubulogenesis
proceeds normally in mice lacking the
proapoptotic factor Bax. Interestingly,
the authors provide compelling evidence
that cystic fibrosis transmembrane con-
ductance regulator (CFTR) acts down-
stream of VIP to promote lumen expan-
sion. Lumen expansion is known to rely
on chloride ion transport mediated by
CFTR. The authors demonstrated that
CFTR is highly expressed in SMG ducts
during lumenogenesis and that pharma-
cological inhibition of CFTR effectively
blocked VIP-dependent lumen expansion
without affecting formation of a contig-
uous lumen. These results place CFTR
downstream of VIP in mediating lumen
expansion, but not lumen formation.

After decades of classical studies doc-
umenting neurotrophic support of periph-
eral neurons by their targets, this study
adds to emerging evidence showing that
neuronal signals reciprocally contribute
to organogenesis (Borden et al., 2013;
Knox et al., 2010). Intriguingly, neuro-
transmitters previously thought to only
modulate adult organ homeostasis are
now being unveiled as instructive cues
during organ development. However,
several key questions remain. How do
derves and target tissues coordinate the
precise timing of their developmental in-
teractions? More broadly, as other epithe-
lial tissues such as pancreas and kidneys
innervated by VIP-positive fibers also un-
dergo lumen formation (Kesavan et al.,
2009; Yang et al., 2013), is innervation a
general regulatory mechanism during tu-
bulation? Additionally, can developmental
cues be harnessed to drive adult tissue
regeneration or lumen formation in trans-
planted organs? In support of this idea,
restoring innervation promotes regenera-
tion of salivary glands damaged by radia-
tion therapy in head and neck cancers
(Knox et al., 2013).

Luminal defects are also associated with
several human conditions such as polycystic
kidney diseases, hypertension,
and epithelial cancers (Andrew and
Ewald, 2010). An important question,
therefore, is whether altered autonomic
innervation may instigate the aberrant
tubular architecture associated with these
pathological conditions. Altogether, the
results reported by Nedvetsky et al., in
combination with other recent studies,
suggest that the autonomic nervous sys-
tem not only contributes to tissue homeo-
stasis as classically viewed but also plays
a significant role in tissue organogene-
sis, regeneration, and possibly disease
pathogenesis.

REFERENCES
Borden, P., Houtz, J., Leach, S.D., and Kuruvilla, R.
Kesavan, G., Sand, F.W., Greiner, T.U., Johans-
son, J.K., Kobberup, S., Wu, X., Brakebusch, C.,
Knox, S.M., Lombaert, I.M., Reed, X., Vitale-Cross,
ence 329, 1645–1647.
Knox, S.M., Lombaert, I.M., Haddox, C.L.,
Abrams, S.R., Cotrim, A., Wilson, A.J., and Hoff-
Nedvetsky, P.I., Emmerson, E., Finley, J., Ettinger,
A., Cruz-Pacheco, N., Prochazka, J., Haddox,
C.L., Northrup, E., Hodges, C., Mostov, K.E.,
Patel, V.N., Rebustini, I.T., and Hoffman, M.P.
Immunol. 24, 574–579.
Yang, Z., Zimmerman, S., Brakeman, P.R., Beau-
doin, G.M., 3rd, Reichardt, L.F., and Marciano,