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Protein tyrosine kinase 7 is essential for tubular morphogenesis of the Wolffian duct

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ABSTRACT

The Wolffian duct, the proximal end of the mesonephric duct, undergoes non-branching morphogenesis to achieve an optimal length and size for sperm maturation. It is important to examine the mechanisms by which the developing mouse Wolffian duct elongates and coils for without proper morphogenesis, male infertility will result. Here we show that highly proliferative epithelial cells divide in a random orientation relative to the elongation axis in the developing Wolffian duct. Convergent extension (CE)-like of cell rearrangements is required for elongating the duct while maintaining a relatively unchanged duct diameter. The Wolffian duct epithelium is planar polarized, which is characterized by oriented cell elongation, oriented cell rearrangements, and polarized activity of regulatory light chain of myosin II. Conditional deletion of protein tyrosine kinase 7 (PTK7), a regulator of planar cell polarity (PCP), from mesoderm results in loss of the PCP characteristics in the Wolffian duct epithelium. Although loss of *Ptk7* does not alter cell proliferation or division orientation, it affects CE and leads to the duct with significantly shortened length, increased diameter, and reduced coiling, which eventually results in loss of sperm motility, a key component of sperm maturation. *In vitro* experiments utilizing inhibitors of myosin II results in reduced elongation and coiling, similar to the phenotype of *Ptk7* knockout. This data suggest that PTK7 signaling through myosin II regulates PCP, which in turn ensures CE-like of cell rearrangements to drive elongation and coiling of the Wolffian duct. Therefore, PTK7 is essential for Wolffian duct morphogenesis and male fertility.

1. Introduction

Tubulogenesis is a highly conserved process, from *Drosophila* to mammals, with each tube having a specific role tailored to the needs of that organ/organism (Andrew and Ewald, 2010; Iruela-Arispe and Beitel, 2013; Lubarsky and Krasnow, 2003). It is clear that the formation of tubes in many tissues arises through a variety of unique processes, *e.g.* wrapping and budding. Once that tube has formed, it then undergoes a series of morphogenic events to generate a tissue/organ of the correct length, shape, and size to fulfill its function. Failure to do so results in the failure of that tissue/organ to function properly. The mesonephric/nephric duct has been well studied from the perspective of kidney morphogenesis (Carroll and Yu, 2012; Karner et al., 2009; Kobayashi et al., 2005; Lienkamp et al., 2012; Schnell and Carroll, 2014), which is an excellent example of branching morphogenesis. However, the cranial portion of the mesonephric duct gives rise to the Wolffian duct, the precursor of the epididymis, which is formed *via* non-branching morphogenesis. Morphogenesis of the Wolffian duct is not trivial in that this duct will eventually elongate to over 1 m in the mouse and 6 m in the human (Hinton et al., 2011), and folds extensively to form an organ of approximately 1 cm and 6–7 cm long, respectively. Undergoing the morphogenic events will ensure that the Wolffian duct is of the proper length and size for sperm maturation, which is critical for male fertility.

Tubular morphogenesis can occur by several mechanisms including cell proliferation, cell rearrangements, cell shape change, and cell recruitment (Andrew and Ewald, 2010). It is unclear which cellular mechanisms underlie Wolffian duct morphogenesis. We hypothesized that cell proliferation was a major contributor (Hinton et al., 2011; Sun and Flickinger, 1982; Xu et al., 2010) and that orientated cell divisions may lengthen the duct and maintain duct diameter. In addition, the Wolffian duct may also elongate *via*

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convergent extension (CE)-like of cell rearrangements and cell shape change. However, cell recruitment is not a major contributor, at least during the embryonic period, because cells identified as originating from the mesenchyme were not observed in the Wolffian duct epithelium (Mugford et al., 2008).

Polarity of cells is often described along the apical–basal axis, but polarity also exists within the plane of the epithelium of that tissue or organ. This type of polarity is referred to as planar cell polarity (PCP). PCP mechanisms play an important role during the development of many organs across many species. Specifically, PCP is required for both CE and oriented cell divisions in several tissues (Karner et al., 2009; Williams et al., 2014; Yen et al., 2009; Yu et al., 2009). Therefore, it is further hypothesized that PCP mechanisms contribute to Wolffian duct morphogenesis.

Protein tyrosine kinase 7 (PTK7), a receptor tyrosine kinase-like molecule, acts as an important regulator of PCP (Hayes et al., 2013; Lu et al., 2004). PTK7 regulates neural tube closure, stereociliary bundle orientation, polarized cell motility, and CE during gastrulation and neurulation (Lu et al., 2004; Williams et al., 2014; Yen et al., 2009). Genetic evidence suggests that PTK7 regulates myosin II activity and PCP orientation in auditory sensory epithelium and neural plate (Andreeva et al., 2014; Lee et al., 2012; Williams et al., 2014). PTK7 is therefore, considered to be a prime candidate in regulating Wolffian duct morphogenesis.

Taken together, our working hypothesis is that PTK7 regulates Wolffian duct morphogenesis through cell proliferation coupled with CE-like of cell rearrangements driven by myosin II activation. This allows the duct to be optimal in length and size for sperm maturation and therefore, male fertility.

2. Results

2.1. *Ptk7* mutant shows abnormal development of the Wolffian duct

Ptk7$^{Xst87/Xst87}$ mice, which are homozygous for the gene-trap allele *Xst87* (Lu et al., 2004), had a shortened Wolffian duct and an unusual coiling pattern compared to controls at embryonic day 18.5 (E18.5) (Fig. 1A and B). Unfortunately, *Ptk7*$^{Xst87/Xst87}$ mice die perinatally due to a craniorachischisis phenotype. Therefore, *T-Cre* (Perantoni et al., 2005) and a "floxed" *Ptk7* allele (Lee et al., 2012) were employed to conditionally knockout (cKO) *Ptk7* from the mesoderm. Pan-mesoderm loss of *Ptk7* (Ptk7$^{flox/Xst87}$:*T-Cre*^{tg}, namely TCre-cKO) resulted in spina bifida in an incompletely penetrant manner. The majority of mice with spina bifida died shortly after birth. However, a significant proportion of TCre-cKO mice did not exhibit spina bifida, but instead had a coiled tail and

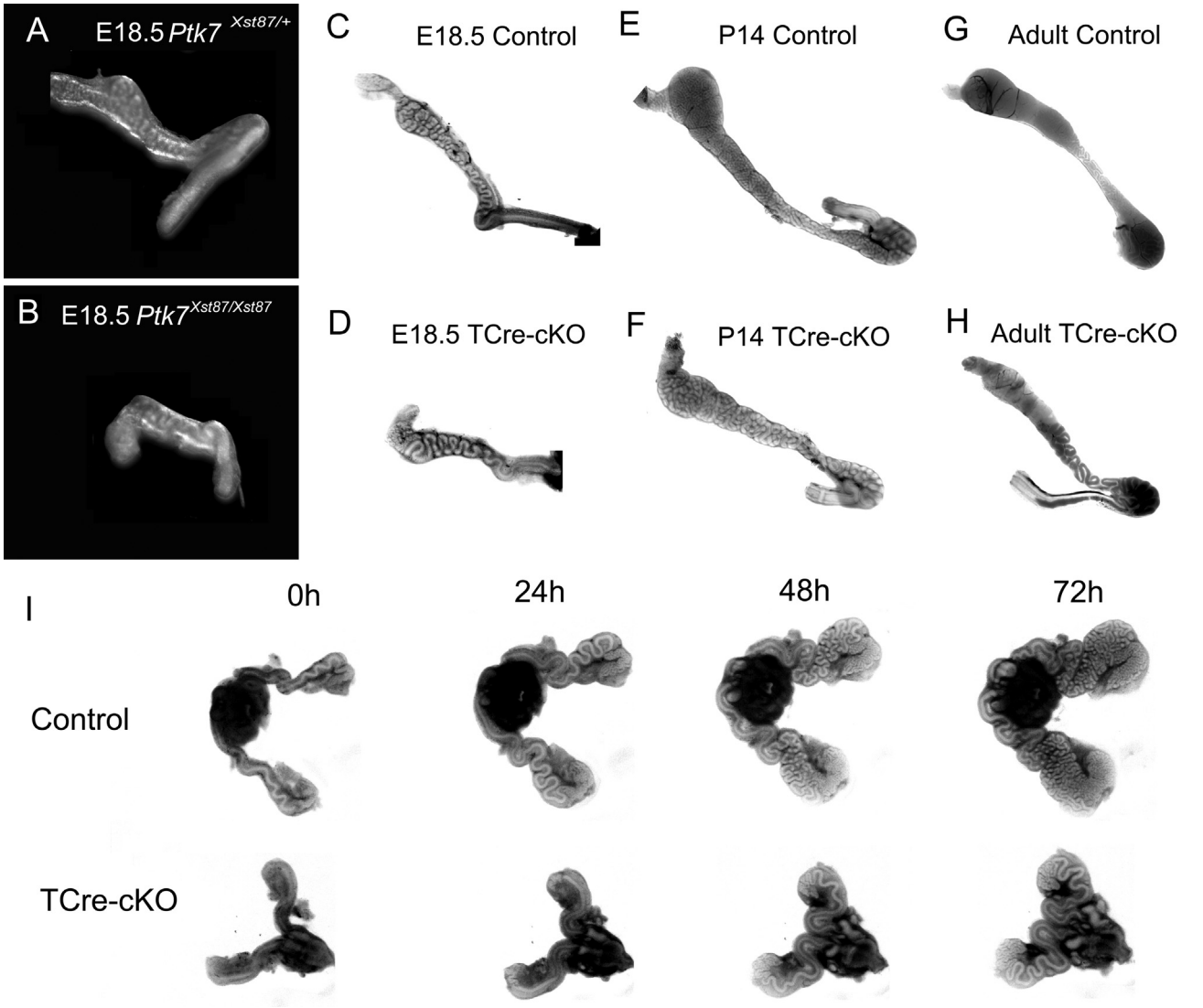

Fig. 1. Abnormal elongation and coiling in *Ptk7* mutant Wolffian ducts. (A, B) E18.5 *Ptk7*$^{Xst87/+}$ and *Ptk7*$^{Xst87/Xst87}$ Wolffian ducts. (C, D) E18.5 control and TCre-cKO Wolffian ducts. (E, F) P14 control and TCre-cKO ducts. (G, H) Adult control and TCre-cKO Wolffian ducts. (I) 72 h of organ culture of E15.5 Wolffian ducts from controls and TCre-cKOs.