

# Sperm Motility Requires Wnt/GSK3 Stabilization of Proteins

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**Inhibition of GSK3 by Wnt signaling stabilizes many cellular proteins, but proof that this effect is independent of  $\beta$ -catenin-mediated transcription is lacking. Koch, Acebron, and colleagues (2015) now demonstrate that transcriptionally silent mammalian sperm require Wnt signaling via exosomes to prevent protein degradation during their lengthy travels through the epididymis.**

Wnt is a growth factor that signals through the inhibition of glycogen synthase kinase 3 (GSK3), one of the main serine/threonine kinases in the cell. Extensive genetic and biochemical studies have demonstrated that the transcriptional activity of Wnt/GSK3 is mediated by the stabilization of  $\beta$ -catenin, a transcriptional co-activator that translocates to the nucleus in response to Wnt signaling, where it binds to TCF/Lef transcription factors (Clevers and Nusse, 2012).  $\beta$ -catenin stabilization is caused by the inhibition of GSK3: when GSK3 is active, it phosphorylates three consecutive sites in  $\beta$ -catenin that define a phosphodegron recognized by an E3 ubiquitin ligase. The ligase then adds Lys-48-linked polyubiquitin chains that specifically target the protein for degradation in proteasomes.

However, GSK3 has many additional substrates. In the human proteome, an amazing 20% of proteins (the GSK3 proteome) contain three or more putative consecutive GSK3 sites, much more than expected by chance alone (Taelman et al., 2010). Wnt likely inhibits many of these possible phosphodegrons, since Wnt3a treatment prolonged the half-life of total cellular proteins by 25% (Taelman et al., 2010). In fact, Wnt-dependent stabilization of proteins (Wnt/STOP) is so extensive that it also increases cell size (Acebron et al., 2014). Using a GSK3 activity biosensor, it was found that this effect of Wnt was maximal at the G2/M phases of the cell cycle, indicating that overall protein catabolism is slowed down as cells prepare to divide into two daughters (Acebron et al., 2014). Given that transcription is inhibited during mitosis, one possibility is that the Wnt/STOP effect is transcription ( $\beta$ -catenin) independent. However, ge-

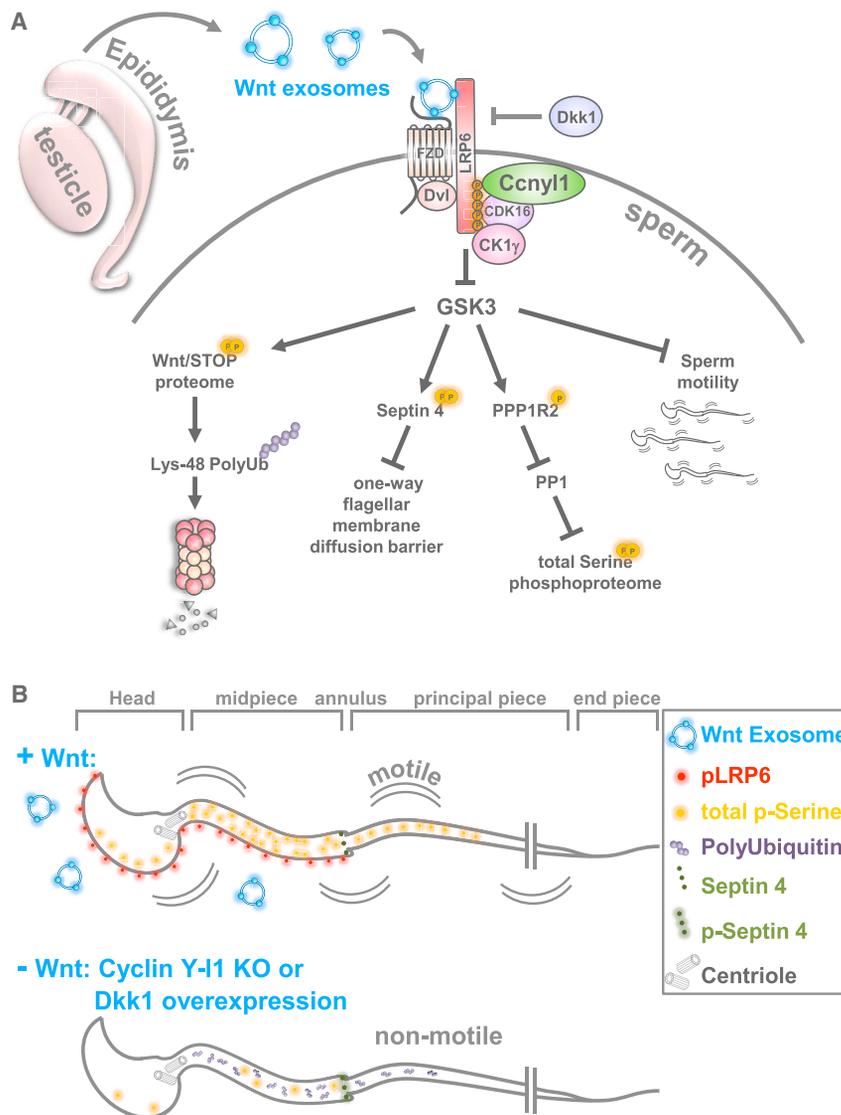
netic proof for this idea is difficult to obtain because  $\beta$ -catenin has multiple functions in cells, for example in cell adhesion. This was the problem Koch et al. (2015) set out to resolve in this study.

Earlier work by Niehrs's group showed that Wnt responsiveness is highest at G2/M because phosphorylation of the Wnt receptor LRP5/6 can be primed by a cyclin-dependent kinase (CDK), which is activated by membrane-bound Cyclin Y expressed specifically at G2/M (Davidson et al., 2009) (Figure 1A). Cyclin Y is ubiquitous and may have pleiotropic effects, but its homolog CyclinY-like1 is only expressed in testis; conveniently, a floxed knockout was available at the European mouse mutant archive. Given the role of Wnt/STOP in maintaining cell size during cell division, one might have predicted defects in sperm formation in this knockout. However, testicular sperm of CyclinY-11<sup>-/-</sup> mice were morphologically normal, perhaps due to residual Cyclin Y activity. Nevertheless, males were 100% sterile, and their sperm were immotile (Koch et al., 2015). Sperm chromatin is extremely condensed, and its histones are substituted by protamines, preventing all transcription. Could it be that cells that can no longer transcribe their genes need to prevent degradation of the proteins they already have? Could Wnt signaling mediate this process?

After sperm exit the testis, they undergo maturation while traveling through a very long (5 to 7 m in man) convoluted tubule called the epididymis (Figure 1). The Niehrs group now reports that the epididymis secretes Wnt exosomes that signal to sperm. Exosomes are small intraluminal vesicles contained within multivesicular bodies (MVBs) that are secreted by fusion to the

plasma membrane. MVBs are involved in both secretion (Gross et al., 2012) and reception (Taelman et al., 2010; Kim et al., 2015) of the Wnt signal in somatic cells. Remarkably, in vitro addition of Wnt3a increased motility and triggered LRP6 phosphorylation in wild-type, but not in CyclinY-11<sup>-/-</sup> mutant sperm (Figure 1B). However, CyclinY-11<sup>-/-</sup> mutant sperm could acquire motility when treated with the GSK3 inhibitor BIO despite being unresponsive to Wnt3a. Furthermore, overexpression of the Wnt antagonist Dkk1 in the epididymis phenocopied CyclinY-11<sup>-/-</sup> mutants and decreased motility. Conversely, sperm motility was increased in mice in which Wnt signaling is elevated by mutation of the  $\beta$ -catenin destruction complex (*APC<sup>min/+</sup>*). In agreement with the lack of transcription in sperm,  $\beta$ -catenin knockout in spermatids produces perfectly motile and fertile sperm (Rivas et al., 2014). These and other data provided very strong evidence that Wnt exosomes are the elusive factors secreted by the epididymis that mediate the maturation and motility acquisition by sperm.

How are these transcription-independent changes in sperm physiology achieved? First, Wnt prevents phosphorylation of the Wnt/STOP proteome. This is reflected by an increase in Lys-48 polyubiquitinated proteins in CyclinY-11<sup>-/-</sup> mutant sperm (Figure 1). In addition, the authors show that levels of many GSK3 substrate proteins are decreased in CyclinY-11<sup>-/-</sup> sperm exiting the epididymis and that this degradation can be prevented by proteasome inhibitors (Koch et al., 2015). Second, Wnt inhibits phosphorylation of Septin 4, a GTP-binding protein that forms filaments around the annulus located between the midpiece



**Figure 1. Post-transcriptional Wnt-STOP in Sperm**

(A) The epididymis secretes Wnt exosome vesicles, which activate Wnt signaling in sperm as they travel through the caput (head region), enabling cell motility. LRP6 receptor is phosphorylated by CDK16, which is activated by CyclinY-like1 (Ccnyl1). In the presence of Wnt, CK1 $\gamma$  phosphorylates LRP6, which then inhibits GSK3 (Zeng et al., 2005). In sperm, there is a broad response to GSK3 inhibition by Wnt that is entirely independent of transcription. (1) GSK3 inhibition stabilizes the Wnt/STOP proteome. (2) Septin 4 phosphorylation by GSK3 inhibits a one-way barrier to protein diffusion along the sperm membrane, resulting in mislocalization of surface proteins. (3) GSK3 activates PP1; in the absence of Wnt, total phospho-serine levels decrease. (4) GSK3 inhibits sperm motility, and Wnt exosomes secreted by the epididymis activate it. (B) In the presence of Wnt exosomes, sperm are motile, LRP6 is phosphorylated, and total phospho-serine is high due to inhibition of PP1. When Wnt signaling is inhibited by CyclinY-I1 mutation or Dkk1 overexpression, sperm are non-motile, Lys48-linked polyubiquitin levels are elevated, total phospho-serine is decreased, and Septin 4 in the annulus is phosphorylated.

and principal piece of the sperm tail (Figure 1B). Septin 4 acts as a one-way barrier or gatekeeper for the diffusion of proteins along the plane of the membrane. When hyper-phosphorylated by GSK3 in CyclinY-I1<sup>-/-</sup> mutants, Septin 4 is inactivated and sperm membrane proteins become mislocalized (Figure 1A). Third,

Wnt decreases GSK3-mediated inhibition of PPP1R2, an inhibitory subunit of protein phosphatase 1 (PP1). PP1 is known to play a key role in repressing sperm motility (Dacheux and Dacheux, 2013). Wnt increases total phospho-serine in sperm through the inhibition of PP1 activity, leading to increased motility (Figure 1B). In an offer-

ing toward translational research, the authors even show that Wnt3a increased motility in two human testicular sperm samples extracted from volunteers during scheduled surgery (Koch et al., 2015).

In sum, this is a very original study on multiple levels. First, it identifies a role for Wnt signaling in sperm maturation. The factors required for sperm motility have been the subject of intensive study, but a role for Wnt had not been previously described (Dacheux and Dacheux, 2013). The discovery that GSK3 inhibition increases sperm motility may have important implications for the treatment of male infertility. Second, it shows that active Wnt exosomes are secreted in a physiological context. It will be interesting to test other bodily fluids for Wnt exosomes (e.g., cerebrospinal fluid and secretions from serous membranes and the female genital tract). Third, it provides genetic proof for a critical role of Cyclin Y in Wnt signaling, reinforcing the concept of cell-cycle control of Wnt receptor activation. Finally, and most importantly, it demonstrates that a rich response to Wnt signaling can be obtained in the absence of transcription, confirming the Wnt/STOP pathway in vivo.

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