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Mini review

# R-spondins: Novel matricellular regulators of the skeleton

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ABSTRACT

R-spondins are a family of four matricellular proteins produced by a variety of cell-types. Structurally, R-spondins contain a TSR1 domain that retains the tryptophan structure and a modified cysteine-rich CSVCTG region. In addition, the R-spondins contain two furin repeats implicated in canonical Wnt signaling. R-spondins positively regulate canonical Wnt signaling by reducing Wnt receptor turnover and thereby increasing beta-catenin stabilization. R-spondins are prominently expressed in the developing skeleton and contribute to limb formation, particularly of the distal digit. Additionally, results suggest that R-spondins may contribute to the maintenance of adult bone mass by regulating osteoblastogenesis and bone formation.



**Contents**



## 1. Introduction

The R-spondins are a family of four secreted proteins in the thrombospondin type 1 repeat (TSR1)-containing protein superfamily. R-spondins are well recognized Wnt signaling agonists. Homologues of R-spondins are present in all vertebrates as well as select invertebrates, such as hemichordates, chordates, and echinoderms, but not *Drosophila* and *Caenorhabditis elegans* (Cruciat and Niehrs, 2013). In mammals, R-spondins have ~60% sequence homology, and all R-spondins have a similar domain structure: an N-terminal putative signal sequence for secretion, two sequential cysteine-rich furin-like domains, a single TSR1, and a positively charged C-terminus (Hankenson et al., 2010; de Lau et al., 2012) (Fig. 1). The TSR1 domain contains conserved tryptophans and a conserved cysteine-rich CSVTCG domain, with the valine and threonine residues replaced with amine containing side-group residues. Specifically each R-spondin has the VT domain replaced with two basic amino acids (either lysine or arginine), one or two polar amino acids (glutamine, asparagine) or in the case of Rspo3 and Rspo4 a single glycine.

Though Rspo3 was originally identified in 1971, as 'thrombin-sensitive protein' (Baenziger et al., 1971), the R-spondin family only began to be elucidated in 2002, with the discovery of Rspo1 (Chen et al., 2002) and subsequent discovery and characterization of the remaining members (Kamata et al., 2004; Kazanskaya et al., 2004; Kim et al., 2006). R-spondins are likely bound to heparan-sulfated proteoglycans in the matrix, as evidenced by their near

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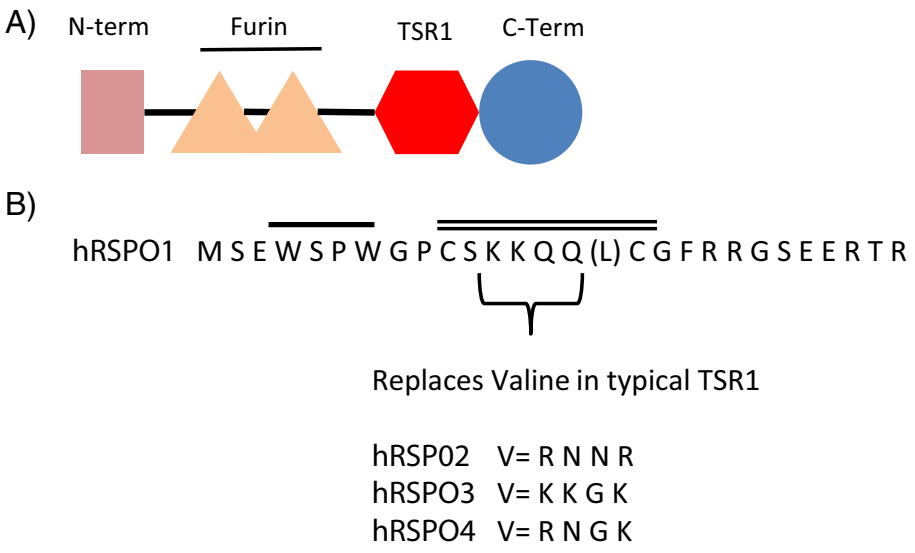


**Fig. 1.** Structure of R-spondin. (A) R-spondins contain an N-terminus that contains a putative secretion signal, two furin repeats in a furin domain, a TSR1 domain, and a C-terminus. (B) The TSR1 domain of R-spondin conserves both the tryptophan rich region (single line) and the cysteine-rich CSVTCG region (double line). The valine in the prototypical TSR1 is replaced in the R-spondins with four amino acids that are basic and polar.

lack of detection in the medium of transfected cells, their subsequent release with added soluble heparin, and their binding to syndecan 4 (Nam et al., 2006; Ohkawara et al., 2011). However, potential interactions with other ECM molecules are not well-described.

The four R-spondin family members can potently activate canonical Wnt signaling in a Wnt-dependent manner, and the furin-like domains are necessary and sufficient for this activity (Kazanskaya et al., 2004; Nam et al., 2006; Kim et al., 2008; Glinka et al., 2011). The mechanism, and more specifically the receptor, for this activation has been highly debated, with reports of Frizzled8, low-density lipoprotein receptor-related protein 6 (LRP6), and Kremen binding to R-spondins. However, none of these studies have been confirmed (Nam et al., 2006; Binnerts et al., 2007; Wei et al., 2007; Glinka et al., 2011; Ohkawara et al., 2011). In 2013, the furin domain of the R-spondins was found to bind to leucine-rich repeat-containing G-protein-coupled receptors 4-6 (LGR4-6) (Chen et al., 2013) after several studies identified them as

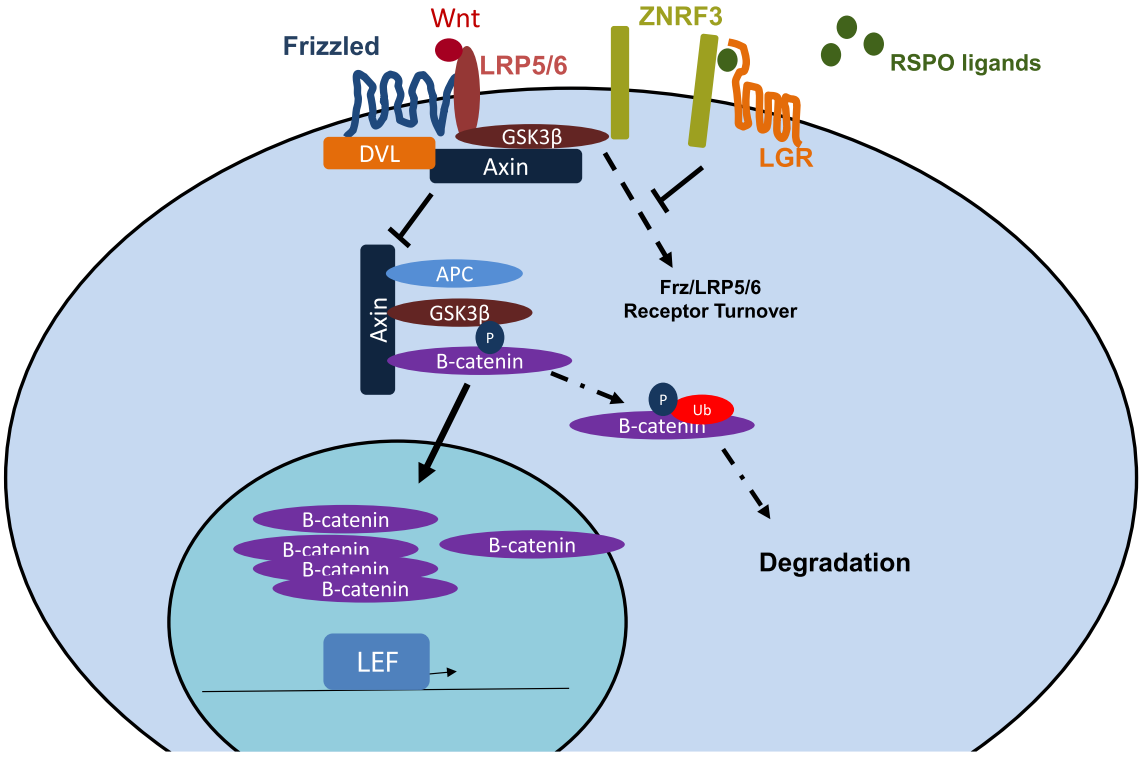


**Fig. 2.** Signaling by R-spondin. This diagram demonstrates the role of R-spondin in regulating canonical Wnt signaling. During the process of canonical Wnt signaling, Wnt binds to Frizzled and LRP5/6. This binding promotes interaction with Axin. When Axin is bound to the receptor complex, this prevents Axin interaction with GSK3beta and APC. GSK3beta is then unable to phosphorylate beta-catenin which results in beta catenin stabilization and nuclear localization to interact with TCF/LEF transcription factors. When GSK3beta is active it results in beta-catenin phosphorylation and increased beta-catenin degradation following ubiquitination and proteasomal degradation. R-spondins regulate this canonical signaling process by inhibiting the action of ZNRF3. ZNRF3 acts as a Wnt signaling inhibitor by promoting LRP/Fzd turnover. When Rspo is bound to a LGR receptor it blocks the turnover promoting activity of ZNRF3.