

P002 Mapping the regions required for Toll and Spaetzle activation

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Drosophila Toll is a class I transmembrane receptor required for dorso-ventral patterning in the embryo and the innate immune response to bacteria and fungi. Toll is activated when a disulphide-linked Spaetzle homodimer is processed, resulting in the crosslinking of two Toll receptors. In its inactive form, homodimeric Spaetzle exists as a precursor that consists of an unstructured pro-domain and a C-terminal region that forms a stable cystine knot (C-106). As the end result of a serine protease cascade, either Easter or SPE will cleave the covalent bond between the pro-domain and C-106, leaving the C-106 motif to bind to Toll. This pro-domain has been shown to be required for proper biosynthesis and secretion of Spaetzle, and remains attached to C-106 non-covalently until the binding event occurs between C-106 and Toll, at which point it dissociates. It therefore appears to be required for part of a negative-feedback and control mechanism for the activation of Toll, as it competes with Toll for binding to C-106. We are currently mapping the interaction regions between C-106 and the Toll ectodomain as well as C-106 and the pro-domain. This is being done via a combination of techniques including molecular modelling, site-directed mutagenesis and electron microscopy.