

The Toll/NF-kappaB pathway: a key to beneficial bacterial symbiosis in cuttlefish reproductive glands

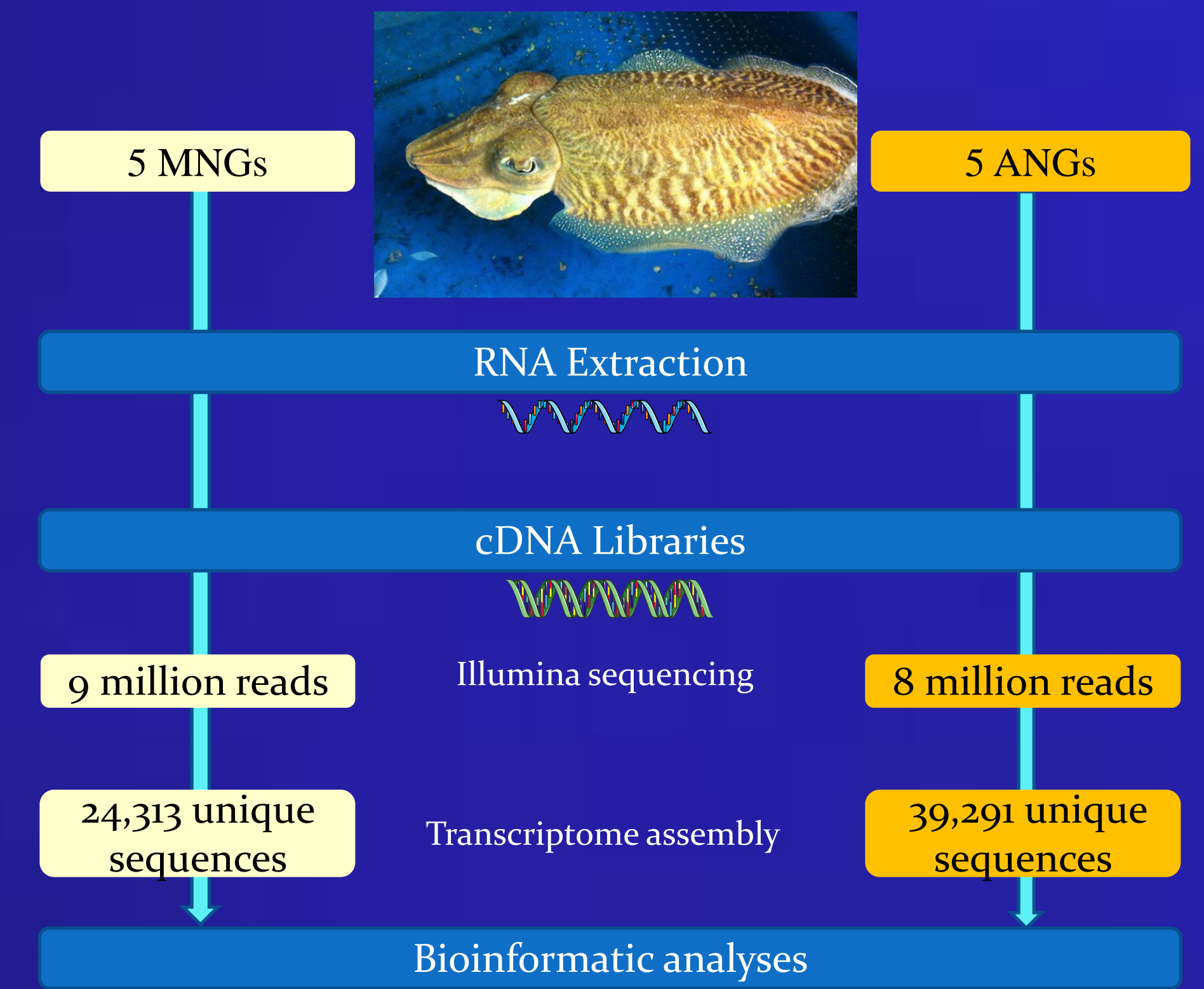
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Co-evolution between animals and bacterial symbionts is widespread among invertebrate and vertebrate species. The beneficial relationship between host and symbiotic bacteria appears to play an important role in host health, and brings benefits in nutrition and immune defense. Sexually mature *Sepia officinalis* females contain two paired nidamental glands involved in egg-laying. The main nidamental gland (MNG) is a non-symbiotic gland that secretes egg case proteins to form a resistant envelope to protect embryos. The accessory nidamental gland (ANG) is a symbiotic gland that harbors a dense consortium of bacteria including *Agrobacterium*, *Roseobacter*, *Sporichthya*, *Rhodobium-Xanthobacter* and *Clostridium*.

Little is known about the mechanisms involved in the initiation and maintenance of symbiosis between cephalopod ANG and the bacterial consortium. Therefore, deciphering symbiotic interactions requires understanding the cross-talk between the symbiotic bacteria and their host. In that respect, the Toll/NF-κB pathway plays a crucial role in the detection of microbial infection and the induction of immune responses.

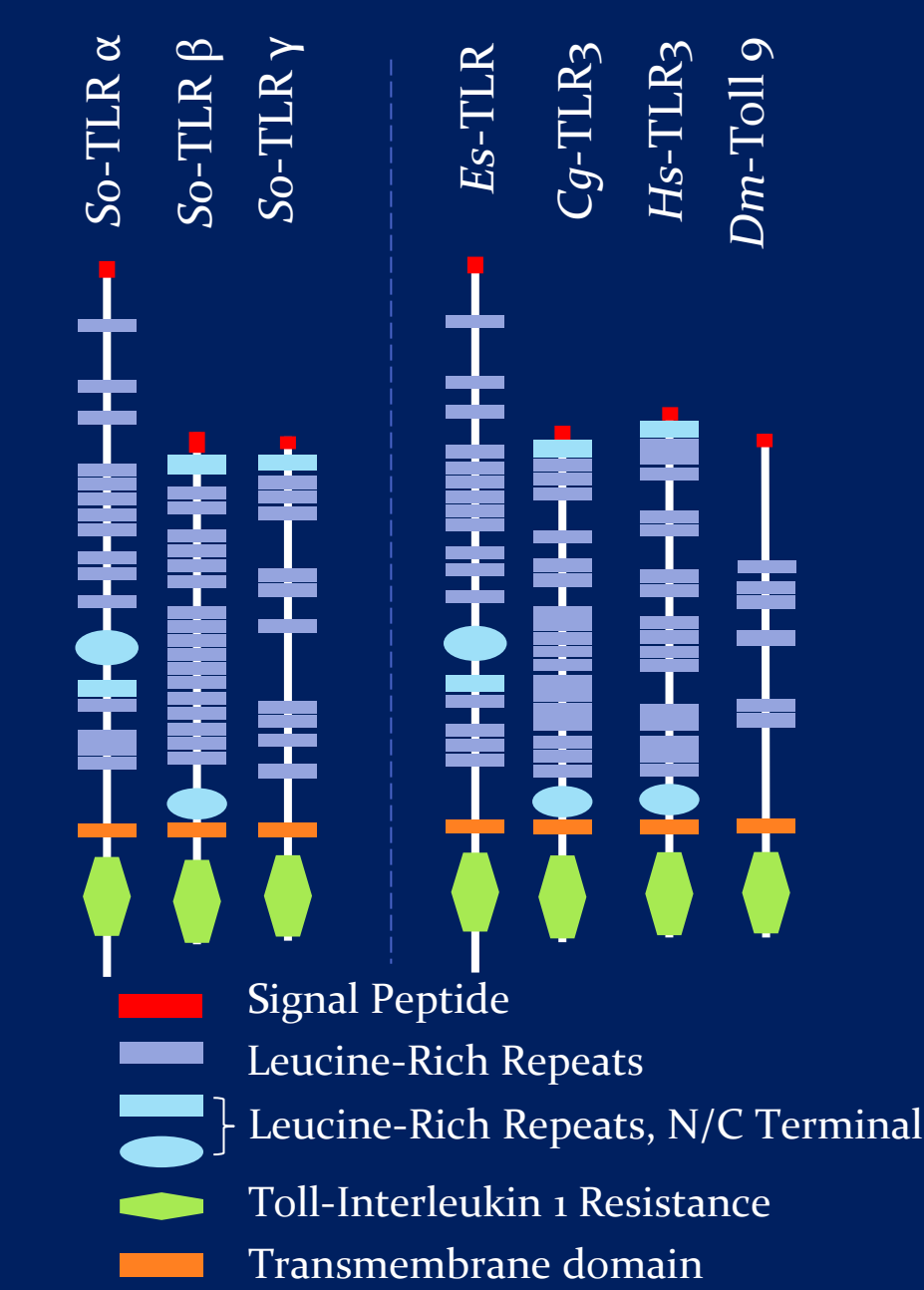
This preliminary study focuses on *Sepia officinalis* reproductive nidamental glands. It aims to describe the innate immune system targeting the Toll/NF-κB pathway in symbiotic and non-symbiotic reproductive glands. To identify transcripts of NF-κB cascade components and assess their expression levels, we used high-throughput sequencing. We compared transcript expression levels in mature reproductive symbiotic accessory nidamental gland and the closely related main nidamental gland that possesses a basal immune state to provide first keys to study how bacterial symbionts can modulate the innate immune system in the accessory nidamental gland.



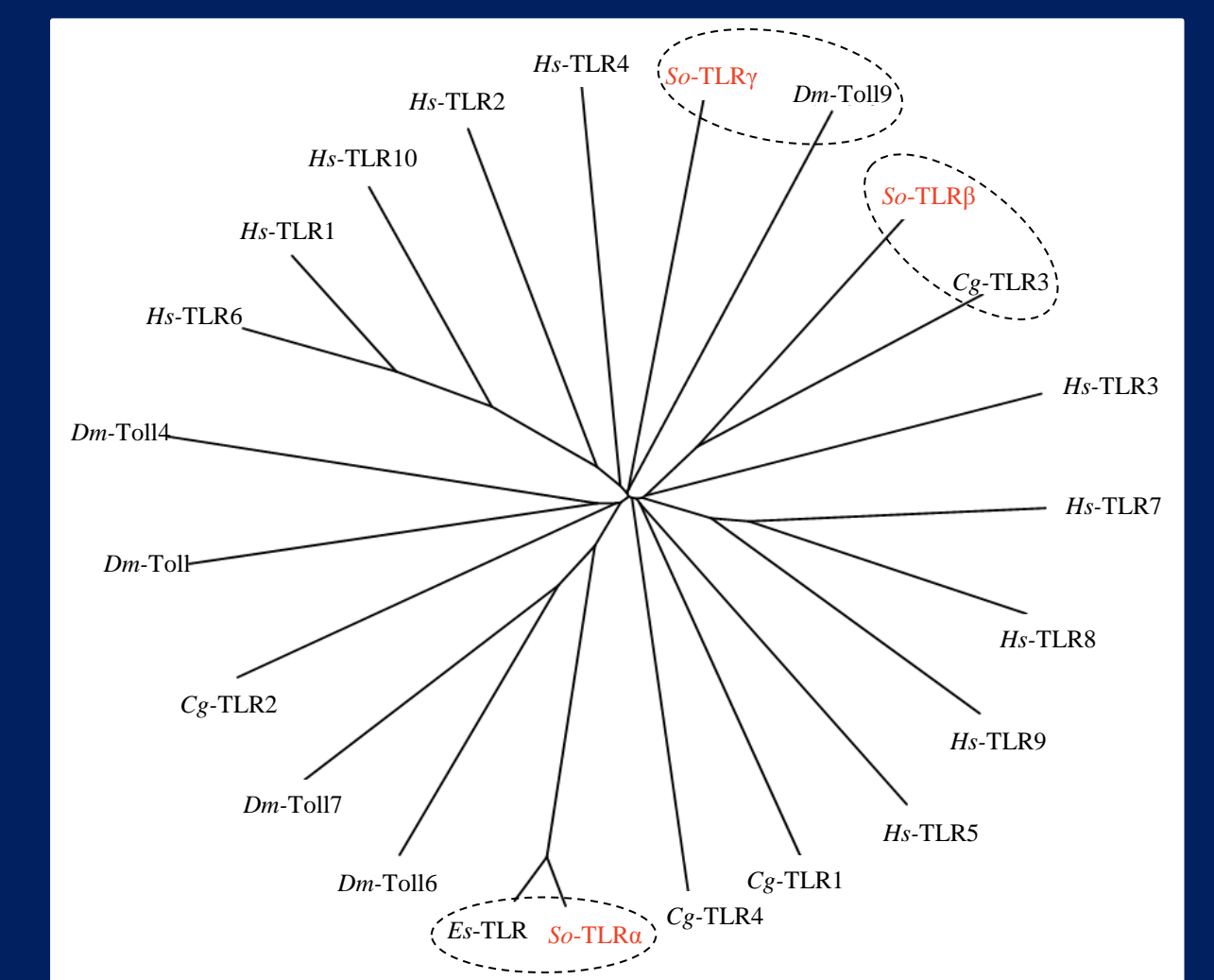
COMPONENTS OF THE NF-κB PATHWAY IN CUTTLEFISH ACCESSORY NIDAMENTAL GLAND

Putative ID	Sequence status	Transcript length	Protein length	Putative function
MAMP-BINDING PROTEINS / IMMUNE RECEPTORS				
Lipopolysaccharide binding protein 2	Full	1302	434	Bacterial lipopolysaccharide binding
Lipopolysaccharide binding protein 3	Full	1611	537	Bacterial lipopolysaccharide binding
Peptidoglycan recognition protein 2	Full	603	201	Bacterial peptidoglycan binding
Peptidoglycan recognition protein 3	Full	687	229	Bacterial peptidoglycan binding
Peptidoglycan recognition protein 4	Partial	789	NA	Bacterial peptidoglycan binding
Toll-like receptor α	Full	3579	1193	Toll signaling protein
Toll-like receptor β	Full	2250	850	Toll signaling protein
Toll-like receptor γ	Full	2499	833	Toll signaling protein
Toll-like receptor δ	Partial	993	NA	Toll signaling protein
Toll-like receptor ε	Partial	966	NA	Toll signaling protein
SIGNALING MOLECULES				
MyD88	Full	1014	338	Toll signaling
IRAK 4	Full	957	319	NF-κB signaling
TRAF 6	Full	1659	553	NF-κB signaling
IκappaB kinase Epsilon	Full	2028	676	NF-κB signaling
IκappaB kinase Gamma	Full	1557	519	NF-κB signaling
Inhibitor of NF-kappaB	Full	1011	337	NF-κB signaling
TRAF 4	Full	1395	465	NF-κB signaling
Calcineurin A	Full	1485	495	Suppressor of NF-κB
Calcineurin B	Full	510	170	Suppressor of NF-κB
REL/NF-kappaB	Full	1413	471	Transcription factor / DNA binding
IMMUE TARGET GENES				
Nitric oxide synthase, inducible	Full	3399	1133	Host immunity
Complement component C3-like protein	Full	5076	1692	Opsonization and phagocytosis

PREDICTED ARCHITECTURES OF TOLL-LIKE RECEPTORS



UNROOTED PHYLOGENETIC TREE OF DIFFERENT TLRs PRODUCED BY THE NEIGHBOR-JOINING METHOD



So: *Sepia officinalis*; Cg: *Crassostrea gigas*; Es: *Euprymna scolopes*; Dm: *Drosophila melanogaster*; Hs: *Homo sapiens*

TRANSCRIPTOMIC ANALYSES:

Identification of the Toll/NF-κB cascade:

- 10 transcripts of Microbe-Associated Molecular Pattern-binding proteins and receptors
- 8 transcripts of signaling molecules from the Toll/NF-κB signaling pathway
- 2 NF-κB target genes

Five Toll-like Receptors in Nidamental glands. So-TLR α, β, γ architectures:

- Integral membrane proteins with:
- N-terminal extracellular LRR domain involved in recognition of bacteria or MAMP-binding proteins
- C-terminal intracellular TIR domain that binds MyD88
- TLRα, only phylogenetically close to *Es*-TLR identified in the symbiotic light organ of a Cephalopod

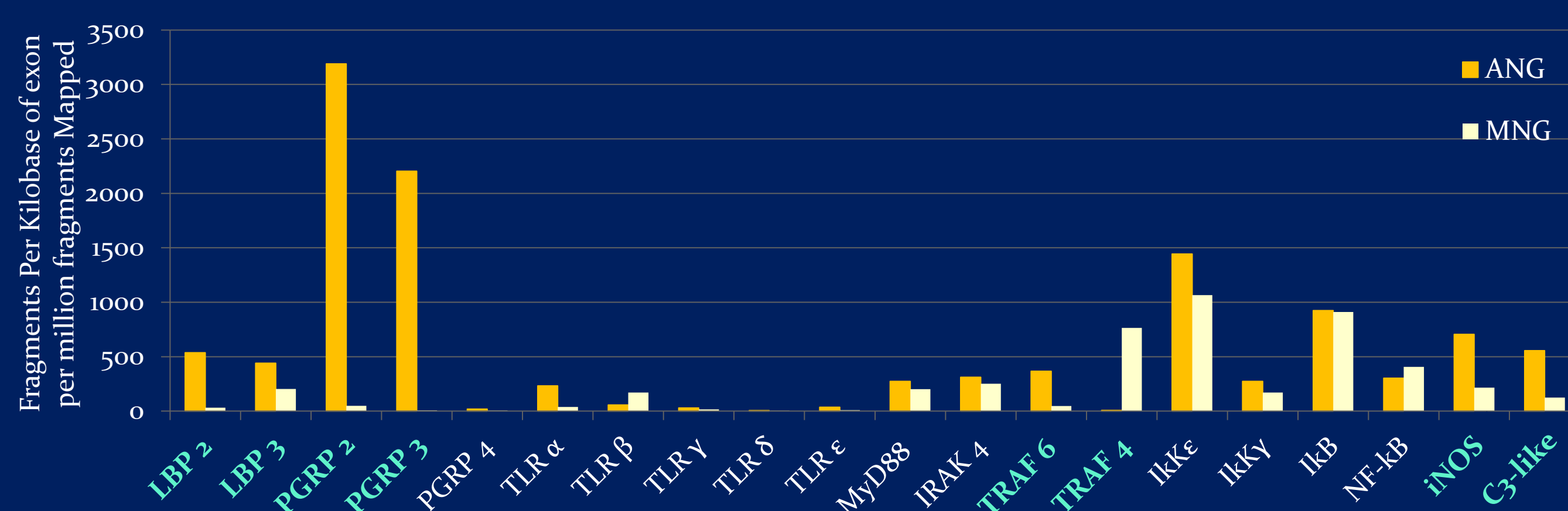
PGRP 2 and PGRP 3: High expression levels in symbiotic ANG, possess highly conserved sequences in their PGRP domain, particularly in substrate/Zn binding and amidase domains.

TRAFs: Low expression level of TRAF4 and high expression level of TRAF6 in ANG as compared to MNG

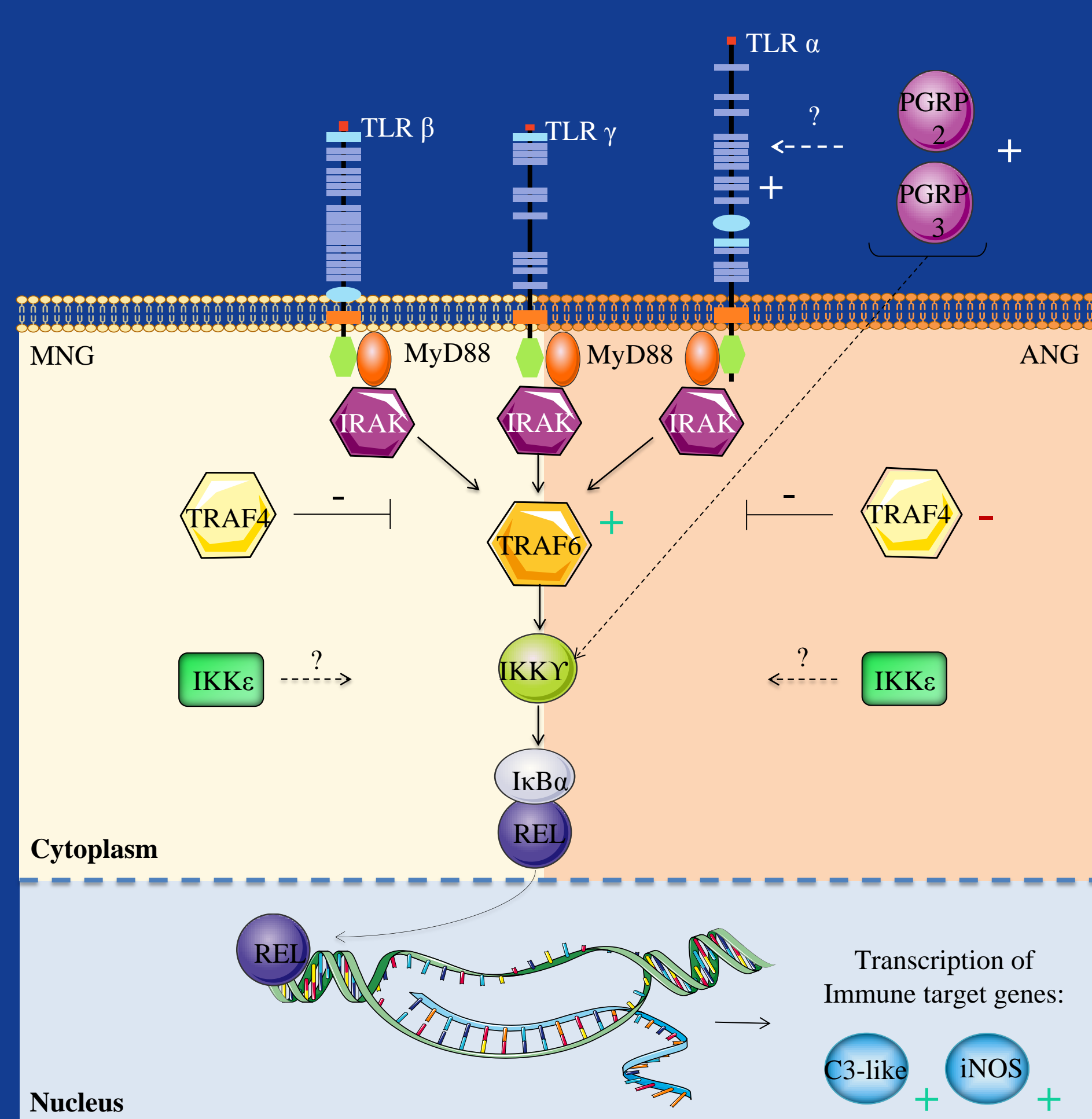
Immune target genes :

- High expression levels of iNOS and Complement component C3-like protein

TRANSCRIPT EXPRESSION LEVELS IN *SEPIA* NIDAMENTAL GLANDS



PUTATIVE TOLL/NF-κB SIGNALING PATHWAY IN *SEPIA* NIDAMENTAL GLANDS



DISCUSSION AND PROSPECTS

This preliminary study describes for the first time an effective Toll/NF-κB pathway in the reproductive main and accessory nidamental glands of *Sepia officinalis*.

The identification of MAMP-binding proteins, of TLRs, of the signaling molecules of the Toll/NF-κB signaling pathway and of immune target genes provides first tools to study the impact of bacterial symbiosis on innate immunity.

Differential transcript expression levels between MNG and ANG suggest an influence of symbiotic bacteria on the Toll/NF-κB pathway. As described in the symbiotic light organ of *E. scolopes*, several immune factors of the NF-κB pathway (PGRPs, iNOS) are involved in orchestrating the initiation and maintenance of *Vibrio* symbiosis (Davidson et al., 2004; Troll et al., 2010).

To confirm and understand the impact of bacterial symbionts on ANG innate immune system, further studies should investigate:

- The role of each Toll-like receptor and MAMP-binding protein in pathogen or symbiont recognition.
- The impact of bacterial symbiosis initiation on the protein phosphorylation cascade
- Transcript expression levels & protein concentrations of inducible NOS, and nitric oxide production during the onset of symbiosis in ANG.

Davidson, S.K., Koropatnick, T.A., Kossmehl, R., Seyoum, L., McFall-Ngai, M.J., 2004. NO means "yes" in the squid-vibrio symbiosis: nitric oxide (NO) during the initial stages of a beneficial association. *Cell. Microbiol.* 6, 139–51.

Troll, J. V., Bent, E.H., Pacquette, N., Wier, A.M., Goldman, W.E., Silverman, N., McFall-Ngai, M.J., 2010. Taming the symbiont for coexistence: a host PGRP neutralizes a bacterial symbiont toxin. *Environ. Microbiol.* 12, 2199–203.