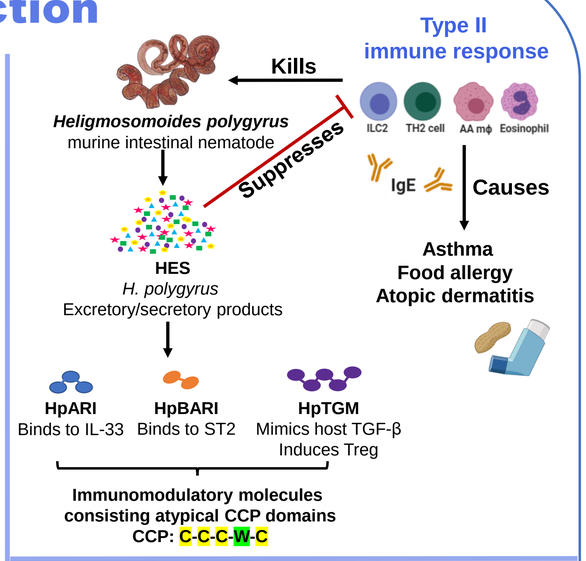
Targeted Identification of New Parasite Immunomodulators Against Allergic Asthma

Vivien Shek[1], Danielle J. Smyth[1], Ruth Savage[1], Henry J. McSorley[1]

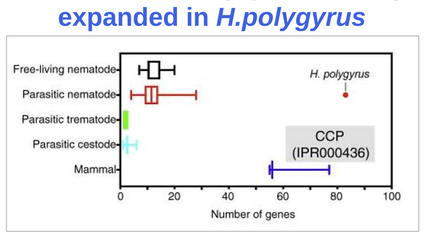
[1]Division of Cell Signalling and Immunology, University of Dundee, UK

Introduction

- Inverse association between some parasite infections and allergic diseases.
- Type 2 immune response is a hallmark of allergic asthma and host defence against helminth infections.
- Molecules secreted by parasites can ameliorate lung inflammation.
- *Heligmosomoides polygyrus* secretes immunomodulatory molecules: HpARI, HpBARI and HpTGM which have shown to suppresses animal models of asthma.
- HpARI, HpBARI and HpTGM all consist of a string of consecutive atypical Complement Control Protein (CCP) domains.



CCP domain-containing proteins is greatly expanded in *H.polygyrus*



(Maizels, Smits and McSorley, 2018)

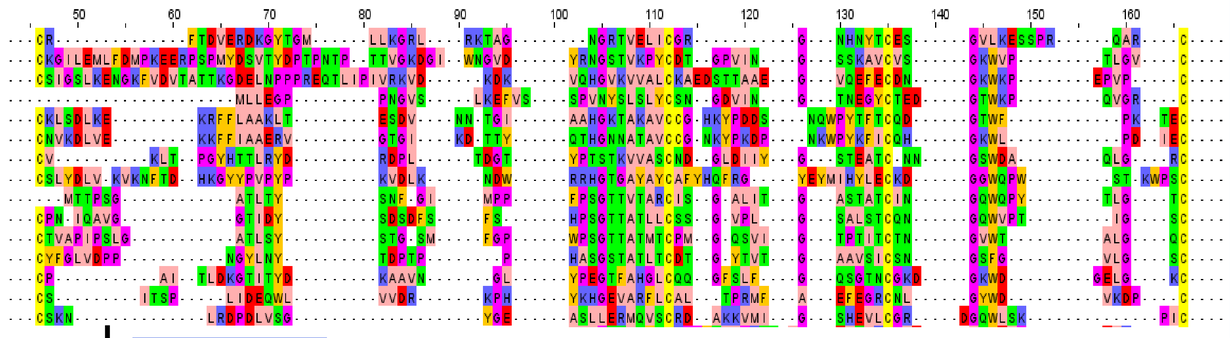
Aim

To use bioinformatic approaches to analyse and identify new parasite immunomodulatory molecules in *H.polygyrus* that contain CCP domains. With the goal to develop a preventative treatment for allergic asthma.

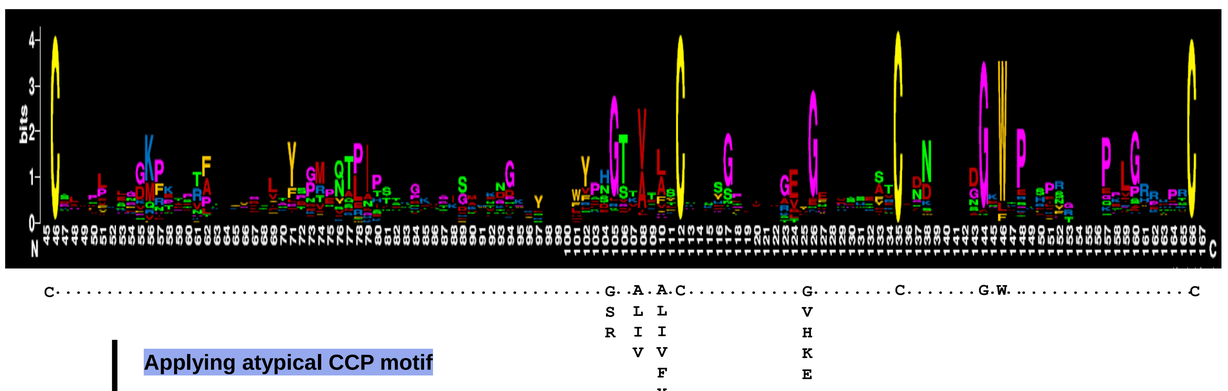
Identifying atypical CCP domain-containing molecules

HpARI, HpBARI, HpTGM and *H.polygyrus* genes annotated as CCP domain (IPR000436)

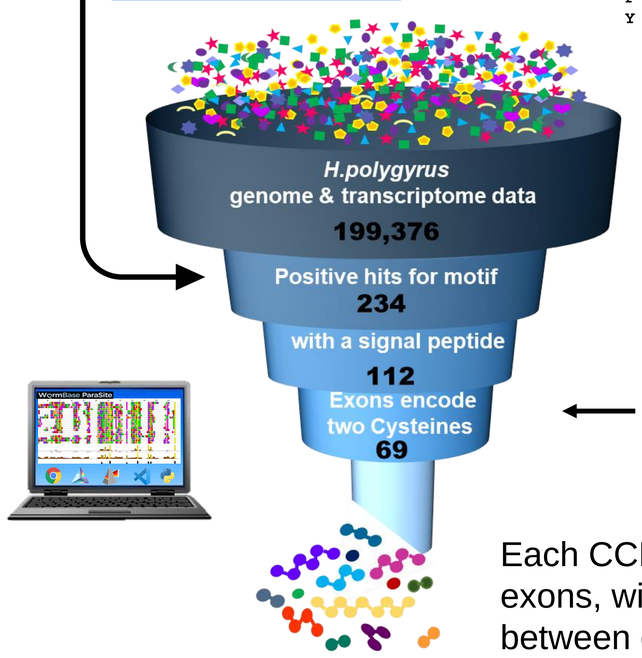
Sequence Alignment



Atypical CCP motif



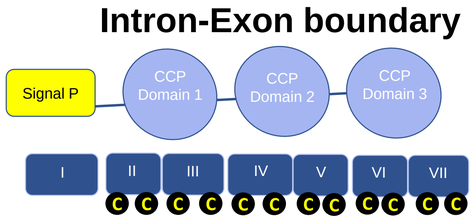
Applying atypical CCP motif



- Protein domain prediction tools - InterPro Scan do not identify HpARI, HpBARI and HpTGM as CCP domain-containing molecules.
- An atypical CCP motif was developed by sequence alignment analyses.

Motif was screened against an in-house *H.polygyrus* transcriptome and genomic data on WormBase ParaSite to predict and identify new atypical CCP domain-containing proteins.

Intron-Exon boundary



Each CCP domain is encoded over two exons, with intron/exon boundaries falling between each CCP domain, this eliminates false positive hits.

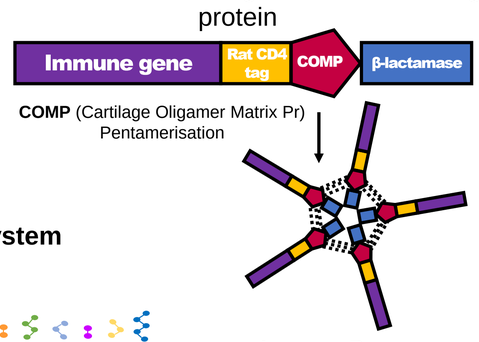
AVEXIS assay for assessing immunomodulation

Avidity-based Extracellular Interaction Screening (AVEXIS) assay, developed by (Kerr and Wright, 2012) was used to screen for interaction of novel parasite molecules with host immune targets.

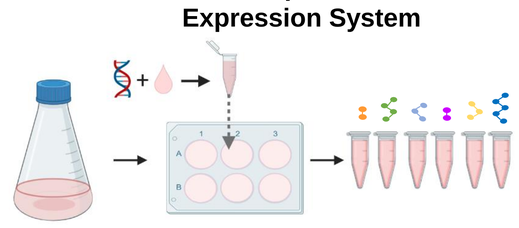
- Parasite molecules were expressed in the BAIT vector.
- A library of immune targets (chemokines, cytokines and their receptors) were expressed in the PREY vector.
- Molecules can be subcloned into either vector.
- Measure interactions in an ELISA-based assay.
- This system is sensitive and has now been optimised to show robust interactions.

AVEXIS BAIT vector to give an N-terminal tagged protein

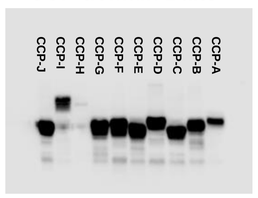
AVEXIS PREY vector to give a C-terminal tagged protein



Expi293 Expression System

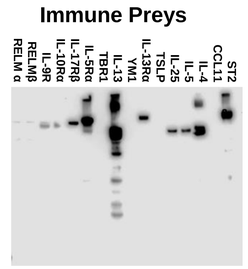


CCP-domain Baits



- Both Baits and Preys are expressed in mammalian cells.
- Crude supernatants containing expressed proteins are used in the AVEXIS assay.

Immune Preys

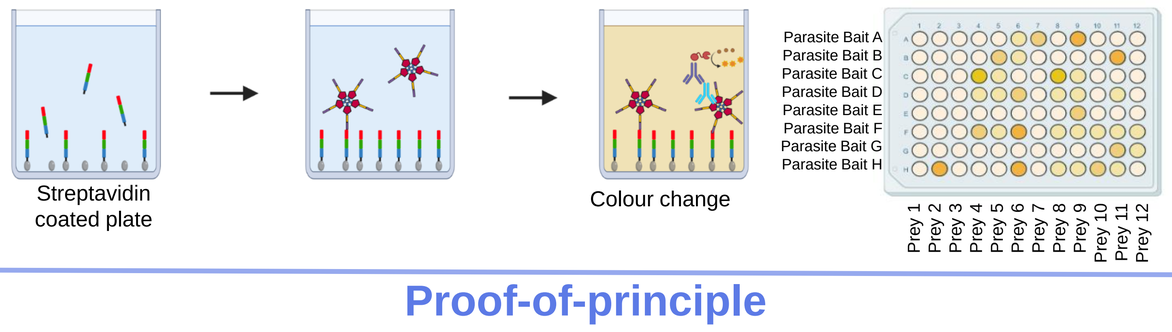


Step 1: Coat ELISA plate with parasite Bait

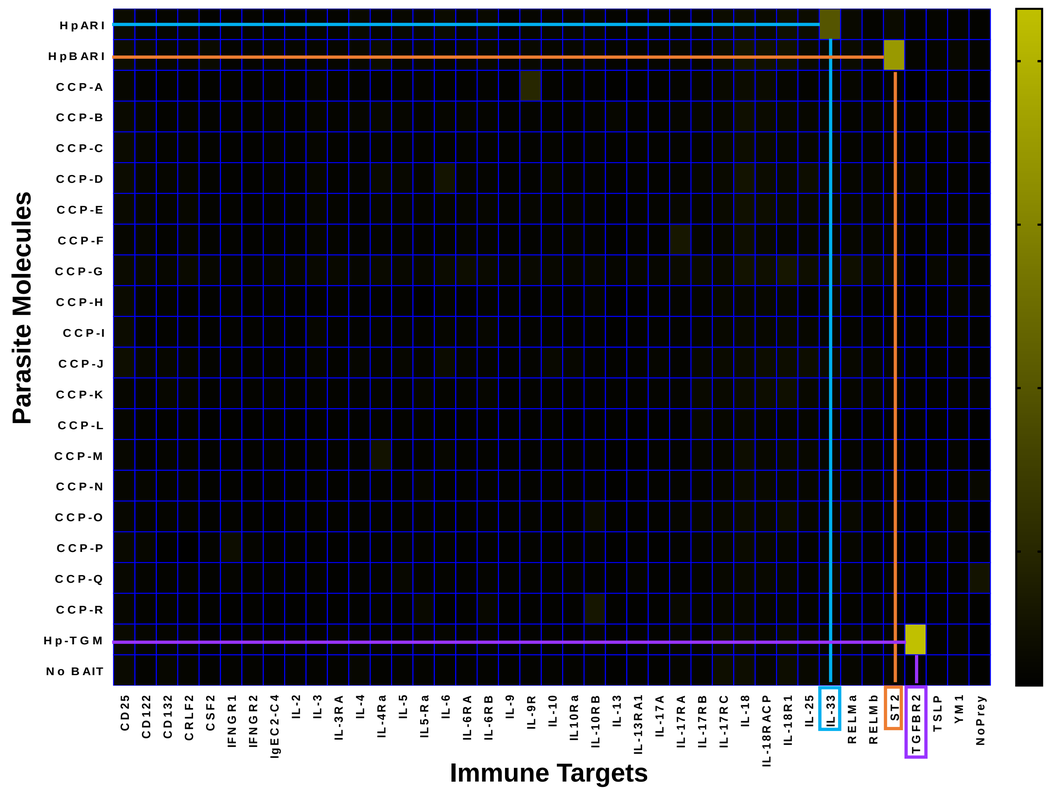
Step 2: Add immune Preys

Step 3: Detect interaction using Anti-β lactamase antibody

Step 4: Read plate at 450 nm



Proof-of-principle



- Proof of concept, interaction is observed between **HpARI**:IL-33; **HpBARI**:ST2 and **HpTGM**:TGFβR2.
- New interaction has been identified but not shown above.

Summary and Next Steps

The methods and analyses described here will aid the development of a larger pipeline in identifying, producing, and characterising new helminth immunomodulatory molecules.

We are now identifying other parasite molecules to screen for novel protein-protein interactions between parasite and host immune targets in the AVEXIS assay.

References

Kerr, J. S., & Wright, G. J. (2012). Avidity-based extracellular interaction screening (AVEXIS) for the scalable detection of low-affinity extracellular receptor-ligand interactions. *Journal of visualized experiments : JoVE*, (61), e3881. https://doi.org/10.3791/3881

Maizels, R. M., Smits, H. H., & McSorley, H. J. (2018). Modulation of Host Immunity by Helminths: The Expanding Repertoire of Parasite Effector Molecules. *Immunity*, *49*(5), 801–818. https://doi.org/10.1016/j.immuni.2018.10.016

School of Life Sciences
University of Dundee

LONG FONDS
Voorheen Astma Fonds