SUMMARY

Title of Thesis: The mechanisms regulating exocytosis of the salivary glands of the soft tick, *Ornithodoros savignyi*

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Numerous bioactive compounds are secreted from large dense core granules in tick salivary glands during feeding. Investigations into the signalling pathways regulating secretion indicated that they are similar for Argasidae (fast feeding ticks) and Ixodidae (slow-feeding ticks). In both cases, dopamine is the external signal that activates adenylyl cyclase, subsequently cyclic AMP levels are increased and Protein Kinase A (PKA) is activated, resulting in the phosphorylation of proteins. Secretion was also found to be highly calcium dependant. Firstly, it requires extracellular calcium (via a L-type voltage-gated calcium channel located on the plasma membrane) and secondly, intracellular calcium which is released presumably in response to IP_3. In contrast to numerous exocrine cells, membrane depolarisation and elevation of the cAMP levels are not sufficient for inducing exocytosis from *O. savignyi* salivary glands. Pathways such as the activation of Phospholipase C, inositol-phosphate kinases, Na^+K^+-ATPases, as well as the disassembly of the actin barrier, have been shown to be essential. Finally, our research also indicated a need for the ATPase NSF, an intact microtubule network and an active cytosolic Phospholipase A_2 for exocytosis. A model has been suggested, but a great deal of research is needed to elucidate all the mechanisms of regulated exocytosis.

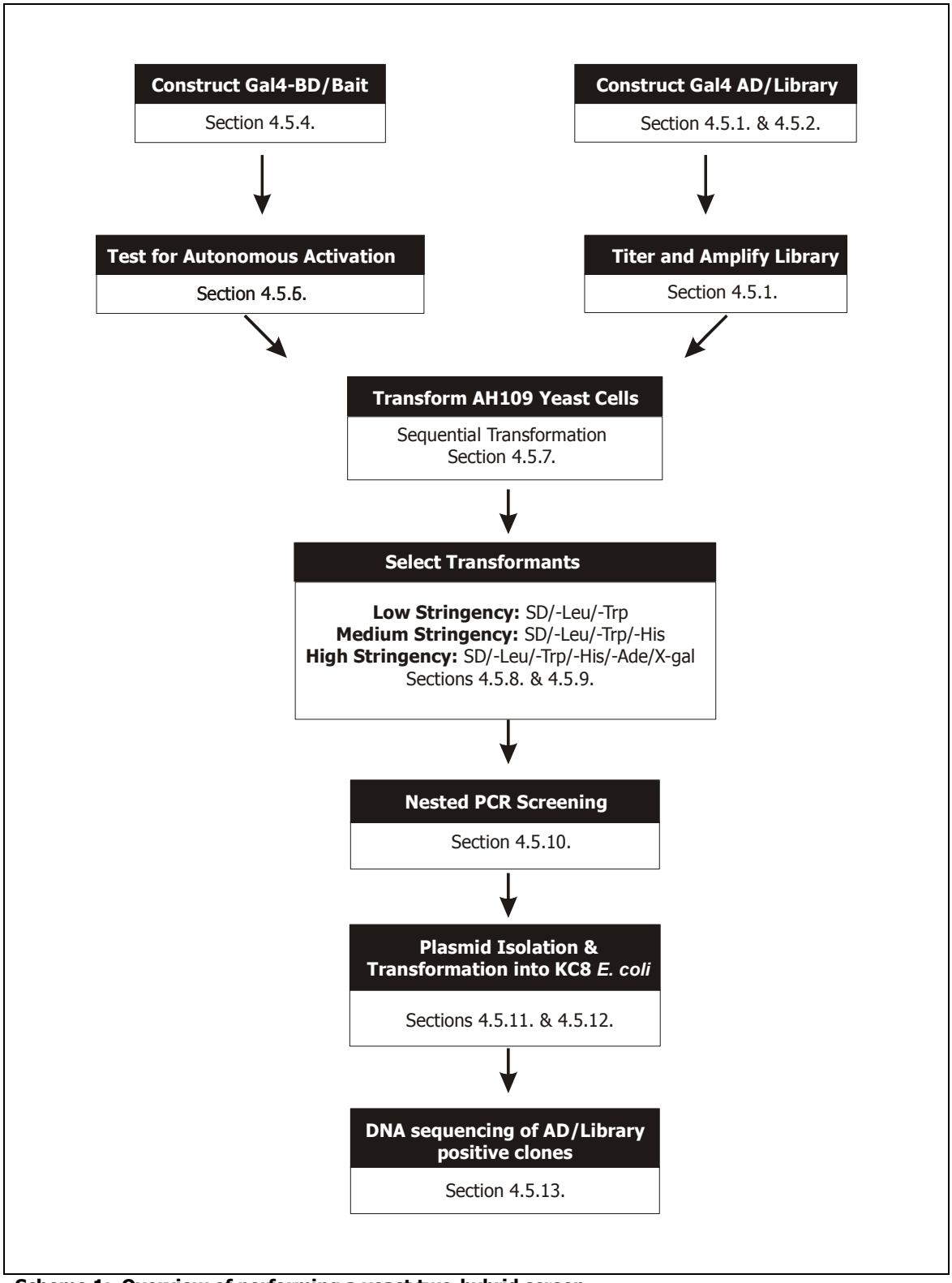
All secretory eukaryotic cells to date require SNARE proteins for fusion of granules with the plasma membrane, leading to the release of granular content. By means of Western blotting we identified the tick homologues of the SNAREs syntaxin, SNAP25 and VAMP, as well as the small GTPase Rab3a, all enriched within the membrane fraction. We also identified the SDS-resistant 20*S* complex, which forms during the docking of granules and is composed of the three SNARE proteins. Confocal microscopy of the SNARE proteins indicates SNAP25 and

VAMP localize to the granule membranes, while syntaxin localises strictly to the plasma membrane.

In order to isolate the tick SNARE homologues we exploited protein-protein interactions by means of the yeast two-hybrid system. Screening of an *O. savignyi* cDNA salivary gland library using rat brain α-SNAP as bait, we identified a transcript encoding a tick syntaxin homologue. It encoded a 126 residue protein which shares 14% identity and 40% similarity with human syntaxin 1. Furthermore, we were able to successfully model the identified protein onto the known crystal structure of human syntaxin 1 and indicate that it shares structural homology with helices 1, 2, 3 and the connecting two loop regions. Following screening of the library with a truncated syntaxin bait construct, two novel domains were identified in all the interacting clones. To date their identity remains unknown.

Functional complementation in the syntaxin knockout yeast strain H603 with an *O. savignyi* cDNA library resulted in the identification of four novel transcripts, which suppressed the temperature sensitive phenotype. Two of these share homology with the N- and C-terminals of syntaxins respectively and were successfully modelled onto the human syntaxin 1 crystal structure. Finally, by exploiting the extensive SNARE binding properties of recombinant rat brain α-SNAP, we were able to isolate the *O. savignyi* SNAREs, i.e. syntaxin, SNAP25 and VAMP, using pull-down assays. These purified proteins will soon be subjected to amino acid sequencing, and their sequences used to confirm the identified transcripts as true syntaxins. By enhancing our understanding of the molecular basis underlying tick feeding, as well as the proteins involved in the processes, we hope to identify possible targets for the rational design of a viable tick vaccine.

APPENDIX



Scheme 1: Overview of performing a yeast two-hybrid screen.