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Haemonchus contortus: Evaluation of two signal sequence trapping systems for detection of secreted molecules ☆

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Abstract

Given that signal sequences between secreted proteins of different species can be interchanged, it is reasonable to expect that both mammalian and yeast signal sequence trapping (SST) systems would secrete *Haemonchus contortus* proteins with similar efficiency and quality. To determine if *H. contortus* cDNAs that contain a signal sequence could re-establish secretion of a reporter protein, mammalian and yeast SST vectors were designed, 10 *H. contortus* genes selected, and their respective cDNAs cloned into these two SST vectors. The selected molecules included genes known to code for excretory/secretory or membrane-bound proteins as potential test 'positives', and genes known to code for non-secreted proteins as test 'negatives'. While differentiation between secretion and non-secretion was evident in both systems, the results indicated greater efficiency was achieved when the mammalian system was used. Therefore, mammalian SST using COS cells would be a more useful tool to screen *H. contortus* cDNA libraries for potential secreted and type-1 integral membrane proteins than yeast SST.
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Index Descriptors and Abbreviations: Signal sequence trapping; *Haemonchus contortus*; Nematode; COS cells; Yeast cells; Secreted proteins; Vaccine target identification

1. Introduction

Gastrointestinal nematodes such as *Haemonchus contortus* are a major cause of lost productivity in the sheep meat and wool industries, particularly in sheep-producing countries in the southern hemisphere (Newton and Meeusen, 2003). Current methods for the control of gastrointestinal nematodes rely heavily on the use of chemicals, but this has resulted in an increase of anthelmintic-resistant parasites and failure to control parasite infections. A potential alternative to chemical control is the vaccination of sheep against parasitic nematodes. Possible vaccine candidates include parasite excretory/secretory (E/S) and surface-localised proteins because they are exposed to the sheep immune system during infection. These secreted and surface proteins are likely to be involved in the establishment and maintenance of the parasite within the host and in the avoidance of the host immune response.

The majority of secreted and type-1 integral membrane proteins have an N-terminal signal peptide that enables the transport of the protein to the extracellular compartment (Nothwehr and Gordon, 1990). Signal peptides have a conserved structure that consists of ~30 amino acids; they are characterised by a basic amino acid at the N-terminus, a hydrophobic centre that varies in amino acid length, and a pair of polar amino acids at the C-terminus (von Heijne, 1985). Signal peptides contain a high level of degeneracy; therefore, identification of expressed sequence tags (ESTs) that code for secreted and type-1 integral membrane proteins is very difficult by bioinformatic analysis of primary sequence in DNA databases, or by hybridisation of DNA probes designed to recognise signal sequences.

☆ *Note:* Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the Accession Nos. M31112, AJ249941, EF636489, AY821552, CB015453, BI595116, CB015409, CB012563, EF636490 and CB012442.

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Although signal sequences are unique, they are largely interchangeable among secreted proteins and even between organisms, with marked similarity between eukaryotic and prokaryotic signal sequences (Jacobs et al., 1997). It is this ability to substitute signal peptides between secreted proteins and still maintain the ability of the chimeric protein to be transported that is utilised in the signal sequence trapping (SST) method.

SST is the selective retrieval of cDNAs that encode E/S and integral membrane proteins. SST in mammalian cells with placental alkaline phosphatase (PLAP) as a reporter gene has been used to detect secreted and transmembrane proteins involved in prostate cancer (Chen and Leder, 1999) and to identify cDNAs that code for secreted and membrane-bound proteins of *Schistosoma mansoni* (Smyth et al., 2003; Pearson et al., 2005). An alternate SST system has been developed in the yeast *Saccharomyces cerevisiae*. With invertase as a reporter gene, this system has been used to identify secreted proteins in rat brain (Klein et al., 1996), secreted proteins in activated mononuclear cells of human peripheral blood (Jacobs et al., 1997) and predicted signal sequence-containing cDNAs from *Theileria parva* (Nene and Bishop, 2001).

In both mammalian and yeast SST systems, secretion of reporter proteins that lack their endogenous signal sequence should be restored by cloning cDNAs that have an initiating methionine and complete signal sequence, in-frame with the reporter gene. To determine if *H. contortus* cDNAs with and without signal sequences could be transported in mammalian and yeast SST systems with similar efficiency, specific vectors were designed and constructed. The mammalian vector contained PLAP as the reporter gene, while the yeast vector had mouse α-amylase as the reporter gene. Both the PLAP and α-amylase reporter genes that were used in vector construction were signalless. Ten *H. contortus* genes were selected and their respective cDNAs cloned into both SST vectors. Selected test clones included genes known to code for E/S or membrane-localised proteins, as 'positive' targets (four molecules) and genes known to code for non-secreted proteins as 'negative' targets (four molecules). Two additional genes were included, a gene that has only a transmembrane domain and no initiating methionine to test the effect of a hydrophobic region on protein translation and secretion, and a known secreted gene that contains a deletion to determine the effect of a frame-shift on reporter gene expression and transport.

2. Materials and methods

2.1. Selection of H. contortus test genes

Haemonchus contortus sequence data (GenBank and in-house) was utilised to select 10 previously characterised *H. contortus* genes that are known or predicted to be positive or negative for secretion (Table 1). The presence of a predicted signal peptide was determined by SignalP V2.0 (http://www.cbs.dtu.dk/services/SignalP-2.0/), iPSORT (http://hc.ims.u-tokyo.ac.jp/iPSORT/) and SecretomeP V2 (http://www.cbs.dtu.dk/services/SecretomeP/).

The four positive clones were: cathepsin B—a cysteine proteinase expressed on the luminal surface of the parasite gut (Skuce et al., 1999); H11—a microsomal aminopeptidase located in the intestinal brush border of adult nematodes (Smith et al., 1997); Hc1—an 'in-house' protein of unknown function with a predicted open reading frame (ORF) which includes a signal sequence; and YC5—a HES15-like 'in-house' protein that has 57% identity at the nucleotide level and 59% identity at the amino acid level.

The four negative clones were: bestrophin—a putative anion channel of unknown function (Sun et al., 2002); caveolin—a proposed plasma membrane scaffold protein involved in the organisation of signalling molecules within the caveolae (Tang et al., 1997); galaptin—a sugar-binding protein (Greenhalgh et al., 1999); and synaptobrevin—a vesicle-associated protein that has been implicated in neurotransmitter release and regulation of fusion events (Nonet et al., 1998).

41B is a novel 'in-house' protein that contains four predicted transmembrane domains and is gut localised as determined by *in situ* hybridisation. The fragment of 41B utilised in this experiment codes for the first predicted transmembrane domain without the initiating methionine. HES15 is a 15 kDa E/S protein that was engineered to delete a single nucleotide at base pair position 386 to result in a frame-shift. The resulting frame-shift alters the amino acid translation from position 130 onward and consequently results in an inactive reporter molecule.

2.2. PLAP vector construction

The mammalian SST vector was produced by modification of the pCMV5 plasmid (Invitrogen) by the strategy originally described by Chen and Leder (1999). Part of the polylinker from HindIII to XbaI, inclusive, was replaced with a 1539 bp PLAP cDNA insert (Fig. 1A). The cDNA insert was produced using Superscript III (Invitrogen) and 1 μg human placental total RNA (BD Biosciences Clontech, BD Smart PCR cDNA Synthesis Kit) according to manufacturers instructions and amplified using gene-specific primers (PLAP-forward 5′-CCCAAGC TTATCATCCCAGTTGAGGAGGAG-3′; PLAP-reverse 5′-ATATCTAGAGGGAGCAGTGGCCGTCTCCAG-3′), to produce a cDNA that lacked the initiating methionine and signal sequence and began with the first amino acid of the predicted mature PLAP (DDBJ/EMBL/GenBank Accession No. M13077, nucleotide 105).

2.3. Amylase vector construction

The yeast SST vector was produced by modification of the pYES2 vector (Invitrogen) by the strategy originally described by Klein et al. (1996) but with α-amylase as the

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