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# Characterization of a *Fasciola gigantica* protein carrying two DM9 domains reveals cellular relocalization property

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ABSTRACT

Even at the present age of whole-organism analysis, e.g., genomics, transcriptomics, and proteomics, the biological roles of many proteins remain unresolved. Classified among the proteins of unknown function is a family of proteins harboring repeats of the DM9 domain, a 60–75 amino acids motif first described in a small number of *Drosophila melanogaster* proteins. Proteins may carry two or more DM9 domains either in combination with other domains or as their sole constituent. Here we have characterized a 16.8 kDa *Fasciola gigantica* protein comprising two tandem repeated DM9 domains (FgDM9-1). The protein was located in the parenchyma of the immature and mature parasite and consequently it was not detected in the ES product of the parasite but only in the whole worm extract. Interestingly, extraction with SDS yielded a substantially higher amount of the protein suggesting association with insoluble cell components. In Sf9 insect cells a heterologously expressed EGFP-FgDM9-1 chimera showed cell-wide distribution but relocated to vesicle-like structures in the cytoplasm after stimulating cellular stress by bacteria, heat shock or chloroquine. These structures did not colocalize with the markers of endocytosis/phagocytosis ubiquitin, RAB7, GABARAP. The same behavior was noted for *Aedes aegypti* PRS1, a homologous mosquito DM9 protein as a positive control while EGFP did not exhibit such relocation in the insect cells. Cross-linking experiments on soluble recombinant FgDM9-1 indicated that the protein can undergo specific oligomerization. It is speculated that proteins carrying the DM9 domain have a role in vesicular transport in flatworms and insects.



## 1. Introduction

Liver flukes in the genus *Fasciola* are large tissue parasites in mammals causing severe pathology of liver parenchyma and the biliary system together with loss of blood in infected animals and humans worldwide (for a recent review on fascioliasis see Ref. [1]). Research on parasite proteins has focused on those with antigenic properties in the infected host assuming that their investigation would result in application for diagnosis, drug, and vaccine development which are the primary targets in the case of human and lifestock parasites. Among these antigens are often highly abundant molecules, e.g. proteases, antioxidant enzymes or surface proteins (for a current overview see Refs. [2,3]). Most of them are classified into well-conserved protein families found across different animal phyla including Chordata and have comparable biological roles. Therefore, cross-reactivity of antibodies or side effects of drugs are a constant concern. In the present research we have investigated a protein that is as far as the detection sensitivity of current sequence comparison algorithms allows to conclude not present in mammals. This is because its function is not required in higher animals or because it has been substituted by other proteins unrelated in their primary sequence. Signature of this protein is the DM9 domain, a repeat motif of unknown function first noticed in the model organism *Drosophila melanogaster* [4]. This approximately 60–75 residues motif is commonly found in Arthropoda, especially insects and is listed in the common protein databases under accessions IPR006616 (InterPro), SM00696 (Smart), DUF3421 (Pfam), and 128937 (CDD). Very few proteins containing the DM9 motif have ever been characterized, and to our best knowledge only two proteins in insects and one in *Fasciola hepatica* have been analyzed to some extent. The *F. hepatica* protein FhTP16.5 was detected in a cDNA library immunoscreen with pooled sera from rabbits infected four weeks with the parasite and the authors of the study

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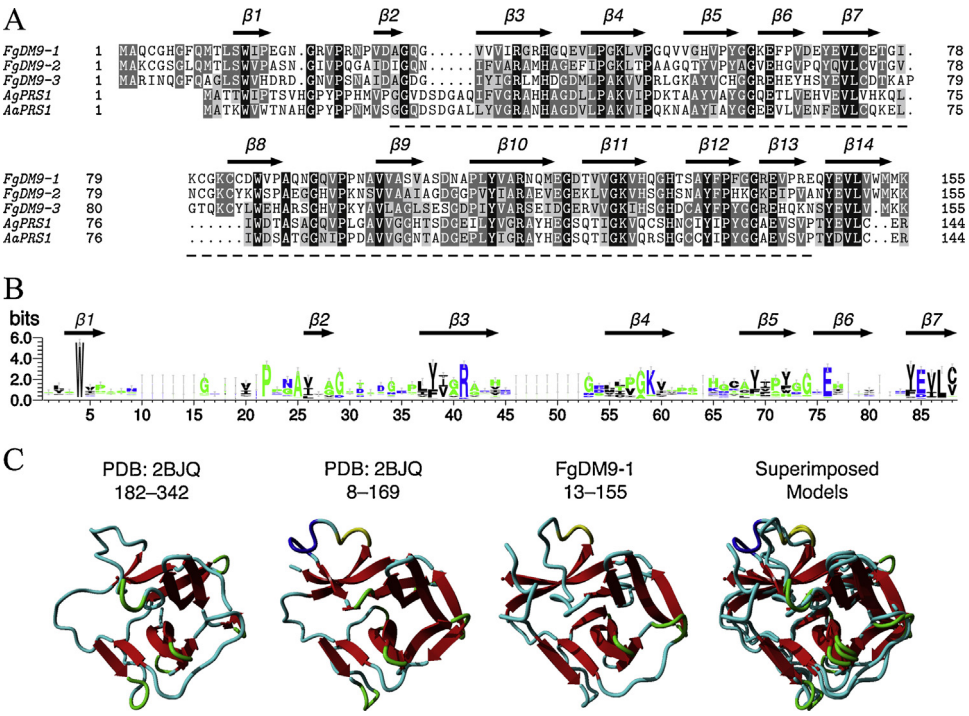


**Fig. 1.** Sequence comparison of FgDM9-1 and sequence logo of the DM9 domain. **Panel A**: Multiple alignment of the amino acid sequences of FgDM9-1, two homologous DM9 proteins from *F. gigantica* FgDM9-2 (GenBank: FN381616), FgDM9-3 (GenBank: FN384711) and PRS1 from *Anopheles gambiae* (UniProt: Q52P90) and *Aedes aegypti* (UniProt: Q1HQX5). Invariable residues are shaded in black and conserved residues are shaded in gray. The predicted β-strand rich secondary structure (PSIPRED 3.3) of FgDM9-1 is indicated by arrows and numbered from β1 to β14. The dashed line indicates the region covered by Pfam DUF3421 (http://pfam.xfam.org/family/DUF3421). **Panel B**: Sequence logo of the DM9 domain calculated from 108 aligned domains from Diptera and Platyhelminthes (supplementary data). Shading by hydrophobicity: RKDENQ, blue, hydrophilic; SGHTAP, green, neutral; YVMCLFIW, black, hydrophobic. **Panel C**: Structure model of FgDM9-1 based on the resolved structure of *A. suum* MFP2a (PDB: 2BJQ). MFP2a domain 2 (amino acids 182–342) was the first ranked template followed by MFP2a domain 1 (amino acids 8–169). Beta strands are shown as red arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

described it as a tegumental antigen with potential application as a diagnostic marker in human fascioliasis [5]. In *D. melanogaster* CG10527 (UniProt: Q8MR08) has been investigated due to a suspected function in the synthesis of juvenile hormone facilitated by a single N-terminal located potential methyl transferase domain [6,7]. The protein carries in addition two DM9 domains and while a null mutant showed no effect on the synthesis of juvenile hormone it seemed to cause a decreased uptake of topically applied methyl farnesoate, juvenile hormone and methoprene. The role of the DM9 domains in the protein was not discussed in the listed publications. In the mosquito *Anopheles gambiae* a protein termed PRS1 comprising two DM9 domains was found upregulated upon infection with *Plasmodium* but also during a standard blood meal. At the RNA level it was abundant in midgut and salivary glands and knock out decreased infection rates with *Plasmodium* [8,9]. The protein showed an interesting relocalization in the cytoplasm upon infection associated with vesicle-like structures in the salivary glands. Our interest in proteins with DM9 domains in trematodes started from a screen of small EST databases predating next generation sequencing projects for potentially abundant parasite antigens in which these proteins were detected. The impact of the much increased trematode molecular sequence date made available in recent times on such in silico findings should not be underestimated [10–15]

## 2. Materials and methods

### 2.1. Parasites

Adult and juvenile (NEJ, 2, 4, 6-week old) *Fasciola gigantica* were collected as previously described [16].

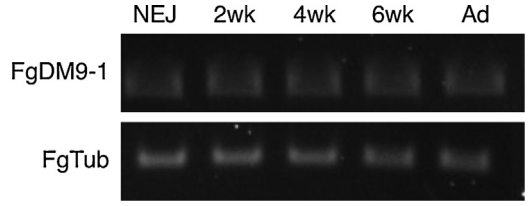


**Fig. 2.** Reverse transcription PCR analysis of FgDM9-1 with total RNA from newly excysted juveniles (NEJ), 2-, 4-, 6-week-old immature worms (2wk, 4wk, 6wk) and adults (Ad). *Fasciola gigantica* tubulin RNA (FgTub) was reverse transcribed in parallel as expression standard.

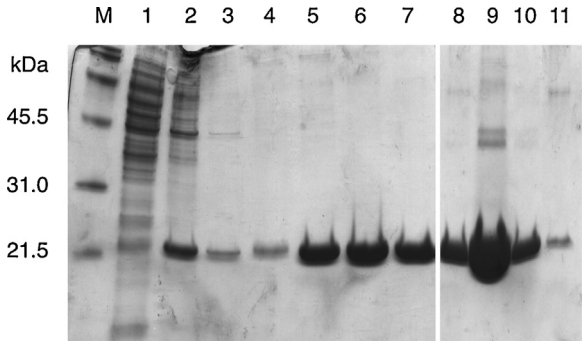


**Fig. 3.** SDS-PAGE showing the purification of recombinant His-tagged FgDM9-1 expressed in *Escherichia coli* by Ni-NTA affinity chromatography under denaturing conditions. Lane 1: flow through, lanes 2–3: wash fractions, lanes 4–7: elution fractions in buffer D, lanes 8–11: elution fractions in buffer E.