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BACTERIAL INFECTION ALTERS PROTEOME OF *CAENORHABDITIS ELEGANS*

Krishnaswamy Balamurugan

Department of Biotechnology, Alagappa University, Karaikudi - 630 003, India

Abstract: *Caenorhabditis elegans* is a suitable animal model for studying various biological events with its immense advantages. Forward and reverse genetic approaches displayed a wide knowledge on the expression profile of various antimicrobial genes on infection by several bacteria. The present era of proteomics exploit this powerful and popular animal model to decipher the proteome level alteration in response to various biological events including host pathogen interactions. Initially, protein work in *C. elegans* focused on biological process that includes various signalling pathways and development related proteins. However, recent initiatives identified the subproteomes and other protein modifications like phosphorylation and glycosylation. Recent studies identified the differences in regulation of proteins against Gram positive and Gram negative pathogens. The present review will bring together results from past proteomic studies in *C. elegans* with special preference to use of *C. elegans* as a model to understand the proteome level changes against bacterial infections.

Keywords: *Caenorhabditis elegans*; antimicrobial genes; Innate immunity; bacterial infection; proteome changes

Introduction

C. elegans is a Diverse Animal Model

C. elegans is a soil nematode, discovered in 1963 by Sydney Brenner. Since its discovery, this animal model has gained attention of scientific community to study various biological events. *C. elegans* is the simplest model system with physiological functions similar to that of humans. Ease of maintenance and growth, very short life span, transparency of organs, a defined genome, first genome to be sequenced among eukaryotes and tractable genetics made *C. elegans* the most suited model to study various biological process (Figure 1). For several years, *C. elegans* has been used as a model to study various bacterial infection. The simple method of feeding based pathogenicity makes the nematode a suitable model for the study. Many human pathogens, including bacteria, fungi and even virus are found to be pathogenic to *C. elegans* (Aballay and Ausubel, 2002; Couillault and Ewbank, 2002; Powell and Ausubel, 2008). Studies with *C. elegans* revealed the virulent factors of bacteria required to cause infection (Dhakal *et al.*, 2006; Kurz *et al.*, 2003) and the possible host response against the bacterial infection (O'Rourke *et al.*, 2006). *C. elegans* has simple immune system (innate) and lack the adaptive immune system, though, the nematode mounts pathogen specific immune responses against different types of infection it encounters. Various genetic tools of present genomic era have made it easy to decipher the worm immunity. The role of putative antimicrobial genes and the signalling pathways through which they are activated are extensively studied in *C. elegans* (O'Rourke *et al.*, 2006; Durai *et al.*, 2011; Kesika *et al.*, 2011 and Sivamaruthi *et al.*, 2011). With all these advantages, *C. elegans* is used as a model for several human disease (Boyd-Kimball *et al.*, 2006), for screening new drugs and to validate drug targets in pharmaceutical industries.

Corresponding Author: **Dr. K. Balamurugan**
E-mail: bsuryar@yahoo.com

Proteome in *C. elegans*

Protein Studies in C. elegans

The proteome study in *C. elegans* began as early as 1975, after a decade of its discovery. The first 2D analysis was performed by O'Farrell who

separated proteins of *C. elegans* using radioactively labelled proteins (O'Farrell, 1975). A well annotated genomic data available for *C. elegans* made it easy to understand various biological events using efficient technologies like microarray, gene silencing and the recent RNA-Seq, a global transcriptome analysis. The proteome of organism is not much easy to study but studying the proteins which regulate most of the biological processes will deliver an important functional insight to the state of organism. Studying proteome provide an accurate level of protein expression in various cells, its interacting partners and post-translational modification which affects the protein function or its localization.

With the development of new technologies like the IPG strips and high-throughput MS analysis and MALDI techniques, it became easy to deduce the proteome of *C. elegans*. Looking back to the protein studies in *C. elegans*, the first report on the age related proteins was in 1994 (Vanfleteren and De Vreese, 1994). Protein profiles of *C. elegans* altered in temperature dependent manner (Madi *et al.*, 2003). Using *glp-1*, a germline proliferation mutant strain, mutation derived changes at protein level was also studied in *C. elegans* (Bantscheff *et al.*, 2004). A sum of 1616 *C. elegans* proteins were identified using ESI-MS/MS analysis providing information on secretary and transmembrane proteins (Mawuenyega *et al.*, 2003). Global changes in worm proteome on infection with the Gram positive bacterium *Staphylococcus aureus* and Gram negative *Aeromonas hydrophila* (Bogaerts *et al.*, 2010) was studied using the 2DIGE and Mass Spectrometry. The results of the study showed the role of metabolic enzymes, energy producers, which are up regulated to fight against infection. The down regulated proteome includes majority of proteins responsible for stress tolerance among which heat shock proteins find special mention. The hypothetical proteins of unidentified function gained much concentration since they are up regulated at certain time point during the infection study. Instead of monitoring the protein changes at one time point, the recent study included four time points to understand the expression of protein after infection and also to elucidate the function of each protein that take part in the immune function and stress tolerance (Bogaerts *et al.*, 2010).

C. elegans Signalling Network Proteome

The *C. elegans* immune system involves various signalling pathways that regulate the expression of immune related genes. At least 5 pathways have been shown to be involved in innate immunity in *C. elegans* and an overlap has been found between the genes involved in pathogen defence against Gram positive and Gram negative pathogens (Figure 2). Infection of C. elegans causes specific changes in gene regulation. In the case of bacterial pathogens that colonize the gut, the induced genes include those encoding antimicrobial peptides and proteins, (e.g., certain caenacins and lysozymes, respectively). Many are specifically expressed in the intestinal cells and these peptides and proteins are secreted into the gut lumen and directly target any infectious micro organisms present at these sites, thereby contributing to the worm's defences (Kurz and Ewbank, 2003). Protein-Protein Interaction (PPI) is an important study in understanding the signalling pathways and communication between proteins. These interactions have their role in both neuronal and metabolic functions (Figure 3). As the transcriptome level do not always correlate with protein level (Gygi *et al.*, 2000), understanding the global interactions between the proteins in *C. elegans* becomes easy with the protein microarray. With the availability of yeast two hybrid system (Walhout *et al.*, 2000) and MS-based peptide mapping, initial attempts were made in identifying the PPI in *C. elegans* (Duchaine *et al.*, 2006). With the advent of the new technologies in proteome studies, understanding the PPI in *C. elegans* will also have impact on validation of mammalian proteome.

C. elegans Evoke Pathogen Specific Immune Response

C. elegans lacks complex adaptive immune system that is mostly responsible for immune specificity. But studies with diverse bacteria provided the corresponding genetic status of the nematode and it is believed that the worm induces pathogen specific immune response(s) which could be specific for either Gram positive or Gram negative bacteria (Alper *et al.*, 2007). Similarly the

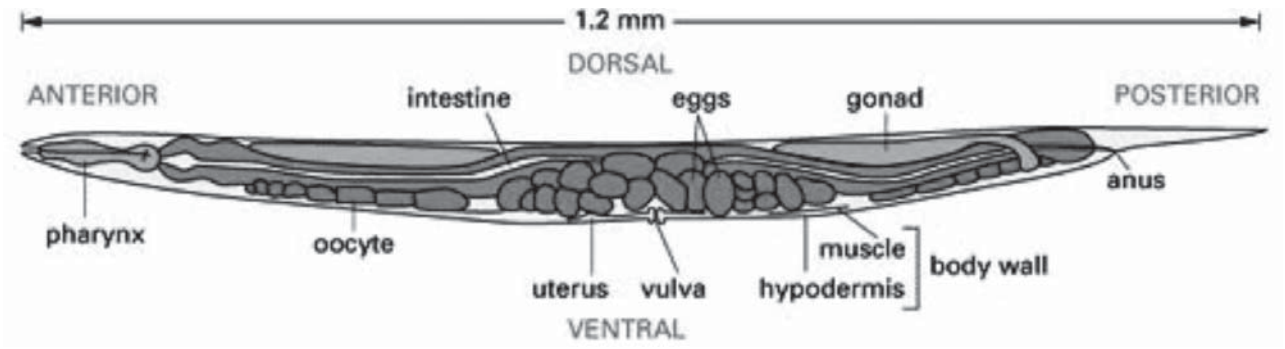

Figure 1: Anatomy of C. elegans

*Adopted from Sulston *et al.*, 1977.

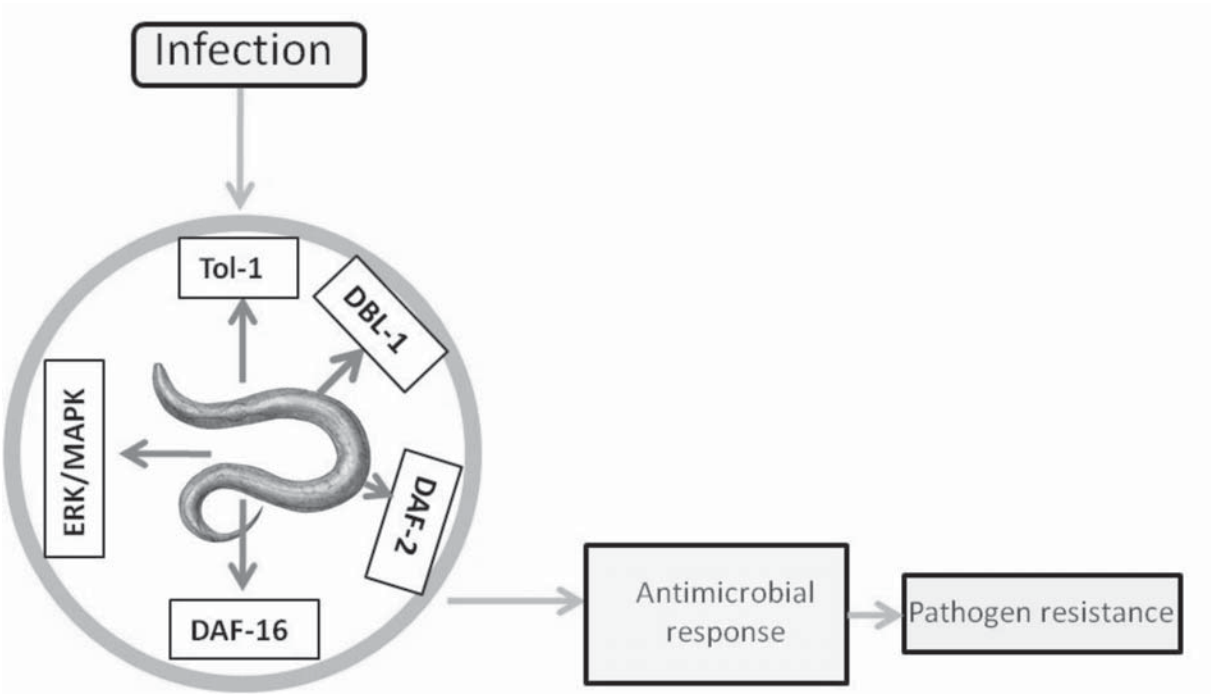

Figure 2: *C. elegans* Immune Pathways

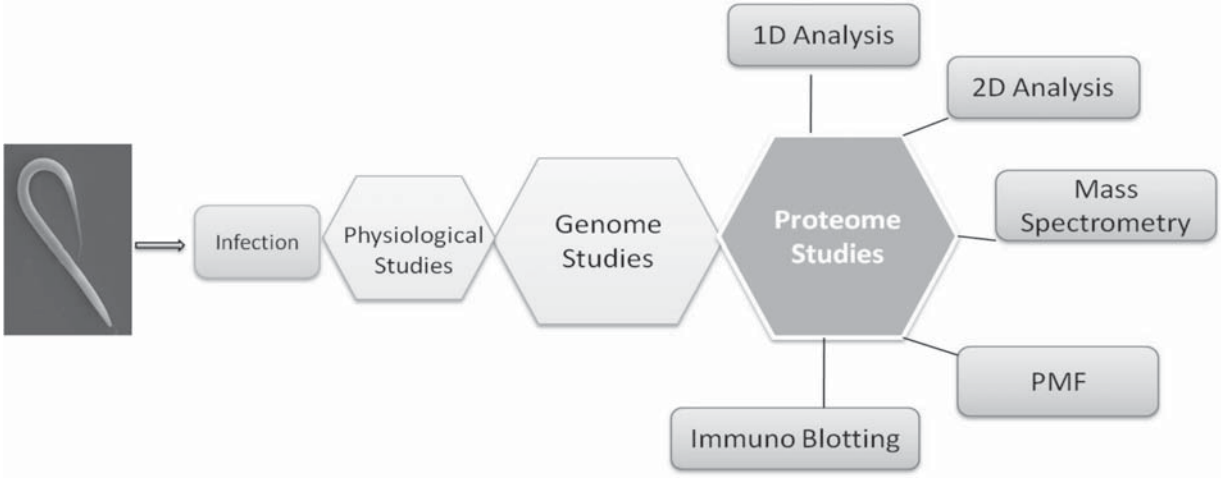

Figure 3: Schematic Representation to Study Proteome Changes in *C. elegans*

proteomic study on infection with the Gram positive and Gram negative bacteria showed a pathogen specific nature of immune response in *C. elegans* (for example the *alh* 8). Genes that are up regulated and believed to have role in the defence system are found to be down regulated at the protein level and function of hypothetical proteins that are up regulated on bacterial infection are yet to be discovered. These results showed that the proteome alteration study in response to bacterial infection in *C. elegans* is important to understand the nature of response from the worm that have greater impact in understanding the mechanism of pathogen evasion and other strategies.

Strategies and Methodologies to Study *C. elegans* Proteome

In order to understand the proteome of *C. elegans* a well designed experiment is important. The nature of pathogen used for study with the time taken for causing lethality and the role of culture medium are the important parameters to be accounted. Once the physiological data and the genetic profile of the infection is understood, studying proteome become meaningful to understand the role of protein that is being regulated in the course of infection. Analyzing at two or more time period during the study is important to understand the complete profile of the changes that occur in the initial stage of infection and during the final state of surrendering to pathogen (Figure 3).

Conclusions

C. elegans is a well characterized organism that have a lot of biological functions similar to humans thus allowing us to infer what might happen to humans. In particular, it is considered to be the best model to study human diseases and understand host pathogen interaction. A complete set of data on *C. elegans* proteome will be of great need to understand the pathological conditions during many bacterial infections. The modification and interaction between proteins are to be focussed on as they have great influence on the development and metabolic pathways involved in *C. elegans*. With the availability of recent proteome tools, understanding the function of proteins involved in immune response against bacterial infections is of hourly need. Studies with different types of pathogens will provide detailed information on the pathogen specific protein alteration(s) in this model system which could be extrapolated to the mammalian systems for analysing their counterpart in future.

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