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Review

Dscam and pancrustacean immune memory – A review of the evidence

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ABSTRACT

Evidence is accumulating for a memory-like phenomenon in the immune defence of invertebrates. *Down syndrome cell adhesion molecule* (*Dscam*) has been proposed as a key candidate for a somatically diversified receptor system in the crustaceans and insects (Pancrustacea) that could enable challenge-specific protection. However, what is the evidence for an involvement of *Dscam* in pancrustacean immune memory, and in particular specificity? Here we review the current state of the art, and discuss hypotheses of how *Dscam* could be involved in immunity. We conclude that while there is increasing evidence for the involvement of *Dscam* in pancrustacean immunity, crucial experiments to address whether it plays a role in specificity upon secondary encounter with a pathogen still remain to be done.

Contents

1. Introduction

1.1. Generating diverse immune receptors and effectors

An effective host immune system is vital to minimise the fitness costs of viral, bacterial and parasite infections (hereafter we use the term 'parasite' in a general sense to include micro- and macroparasites). Parasites represent a co-evolving and heterogenous selection pressure for immune systems, to which hosts have responded with the evolution of a variety of recognition and effector molecules, enabling resistance or tolerance of the parasite (Raberg et al., 2009; Schmid-Hempel, 2011). Broadly speaking, immune receptor and effector diversity is accomplished via diversity at the DNA level and somatically generated diversity (Du Pasquier, 2005, 2006). Jawed vertebrates have responded to the challenge of parasite recognition and elimination by evolving, for example, multigene families such as the major histocompatibility complex (MHC) and somatic recombination of variable-diverse-joining, V(D)J, gene segments (Du Pasquier, 2006). These processes of diversity generation have resulted in highly specific immunological memory, a characteristic trait of vertebrate immunity.

Invertebrates on the other hand do not have the adaptive immune machinery, such as clonal expansion of antigen-specific lymphocytes (Murphy, 2011), to generate a broad defence capability. The insect immune repertoire, is generally thought to rely on less diverse receptors and effectors, such as the multigene family of peptidoglycan recognition proteins (PGRPs) with 13 representatives in *Drosophila melanogaster* (Lemaitre and Hoffmann, 2007),

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or somatic diversification via alternative splicing of PGRP-LC resulting in three different extracellular domains (Werner et al., 2003). However, a putative candidate for generating immunologically diverse receptors/effectors has emerged over the last few years, with the discovery of an immune role for *Down syndrome cell adhesion molecule* (*Dscam*) (Watson et al., 2005). This gene combines both genotypic and somatic variation to create diversity. Via mutually exclusive alternative splicing in the ecto- and transmembrane-domains, and exon skipping in the endodomain, the *D. melanogaster Dscam* gene can potentially produce more than 152,000 isoforms (Yu et al., 2009; Fig. 1). The more commonly referenced number for *D. melanogaster*, more than 18,000 isoforms, refers to only the extracellular isoforms (Sun et al., 2013; Watson et al., 2005), which are the domains that cause *Dscam's* recognition specificity (Sun et al., 2013). The corresponding number for the mosquito *Anopheles gambiae* is nearly 16,000 isoforms (Dong et al., 2006). Although these numbers are many orders of magnitude lower than the diversity of B/T cell receptors and antibodies found in vertebrates (Murphy, 2011), the diversity would be unprecedented compared to known insect immune effectors. Alternatively spliced *Dscam* has since been discovered in other insect and crustacean genomes (Brites et al., 2008; Chou et al., 2009; Crayton et al., 2006; Watthanasurorot et al., 2011), and it has been implicated in the immune response of a number of pancrustaceans. Brites et al. (2013) suggested that *Dscam* has convergently diversified across the arthropods: they found that representatives from two arthropod taxa outside of the Pancrustacea, the Myriapoda and Chelicerata, have diversified *Dscam* via whole gene duplications, with some immunoglobulin domain duplication also found in the myriapod (Brites et al., 2013). Because of the recent nature of this discovery, limited data exist examining *Dscam* in these taxa in relation to immune defence, therefore for the purposes of this review we will focus on pancrustacean *Dscam*.

1.2. Dscam diversity and its role in the nervous system

DSCAM was first discovered in humans, acquiring its name after being mapped to a Down syndrome region on chromosome band 21q22 (Yamakawa et al., 1998). A homolog of *DSCAM* was subsequently found in *D. melanogaster*, and was named *Dscam* (Schmucker et al., 2000). The origin of the *Dscam*/*DSCAM* gene probably lies after the split between the Cnidaria and the Bilateria (Armitage et al., 2012; Brites et al., 2008; Crayton et al., 2006) more than 600 million years ago (Peterson et al., 2008). *DSCAM* and *Dscam* are members of the Immunoglobulin (Ig) superfamily, the largest group of related cell surface proteins (Schmucker and Chen, 2009), and although they share the same protein domain structure (Schmucker and Chen, 2009) the vertebrate gene has only two predicted isoforms (Yamakawa et al., 1998). The conserved domain structure consists of ten Ig domains, six fibronectin type III (FN) repeats, a transmembrane domain and a C-terminal cytoplasmic tail (Schmucker et al., 2000) (Fig. 1A). Mutually exclusive alternative splicing of pre-mRNA results in mRNA with both constant and variable exons (Fig. 1B), and the potential to generate a multiplicity of isoforms. The four variable regions in *D. melanogaster* are the extracellular exon clusters 4, 6, 9 and the transmembrane domain exon 17, with 12, 48, 33 and 2 alternatively spliced exons respectively (Fig. 1C). *Dscam* was initially found to be vital for the nervous system, where its role has mainly been explored in *D. melanogaster*: it plays an essential role in neuronal wiring by mediating the process of self avoidance. In a developing fly brain, neurons generate a unique pattern of *Dscam* isoforms on their cell membrane that allows the neuron to discriminate between its own neurites (self) and neurites of another neuron (non-self) (Zipursky and Grueber, 2013). It has been shown that the homophilic binding of two identical extracellular domains of Dscam leads to a cellular domain mediated repulsion of the neurons. The intracellular pathways that trigger this repulsion remain unknown. However, heterophilic binding of two different extracellular domains of Dscam results in tiling of two neurons (Hughes et al., 2007; Matthews et al., 2007; Soba et al., 2007). Furthermore, Wu and co-workers (2012) showed that binding of two *Dscam* isoforms, where only two of three variable extracellular domains of Dscam are identical, already results in a weak but efficient repulsion signal. To produce such a variety of neuron identities in order to not activate unwanted repulsion, these thousands of *Dscam* isoforms are essential (Forbes et al., 2011; Hattori et al., 2009).

1.3. First evidence of an immune role for Dscam

In 2004 Neves et al. found that individual haemocytes can each express at least two of each of the isoforms from each Ig domain (see Table 1 and Fig. 2 for a general overview of *Dscam's* role in pancrustacean immunity). One year later, by examining expression

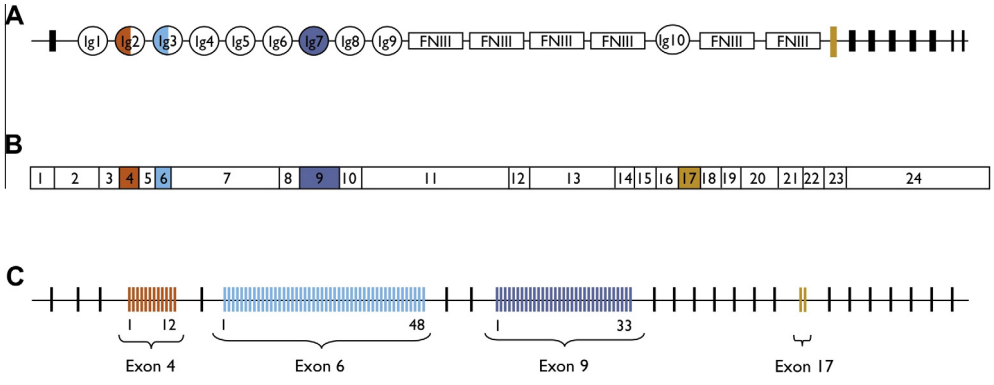

Fig. 1. Dscam protein, mRNA and genomic structure. (A) Dscam protein structure for *D. melanogaster*. The alternatively spliced exons encode the N-terminal half of Ig2 (exon 4 in *Drosophila*; red semicircle); the N-terminal half of Ig3 (exon 6 in *Drosophila*; light blue semicircle), all of Ig7 (exon 9 in *Drosophila*; dark blue circle), and the transmembrane domain (Exon 17 in *Drosophila*; orange rectangle). (B) *Dscam* mRNA. Constant exons are shown as white boxes. Exons that undergo mutually exclusive alternative splicing follow the same colour scheme as for the protein structure. Endodomain exons 19 and 23 can be contained or lacking (Yu et al., 2009), which increases the number of potential isoforms to 4 × 38,016 = 152,064. (C) *Dscam* genomic DNA for *D. melanogaster*. The gene consists of 20 constant exons (shown as black lines), mutually exclusive alternative splicing occurs for exons 4, 6, 9 and 17; one of 12 exon 4 alternatives, one of 48 exon 6 alternatives, one of 33 exon 9 alternatives and one of two exon 17 alternatives are present in each mRNA. This enables the vast number of 12 × 48 × 33 × 2 = 38,016 potential splice variants. However, this number may be an overestimate because exon 6.11 is not expressed (Neves et al., 2004; Sun et al., 2013; Watson et al., 2005) and has since been described as a pseudo-exon (Sun et al., 2013). Exons 19 and 23 can be contained or lacking, increasing the number of potential isoforms to more than 152,000 (Yu et al., 2009). (Figure after Schmucker et al., 2000 and Armitage et al., 2012).