The Potential Consequences for Cell Signaling by a Class of NOD-Like Receptor Proteins (NLRs) Bearing an N-terminal Signal Sequence

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Received date: April 17, 2017; Accepted date: May 10, 2017; Published date: May 19, 2017

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Commentary

Toll-like and NOD-like receptors (TLRs/NLRs) recognise pathogen- or damage-associated molecular patterns (PAMPs/DAMPs) and are key initiators of cell signalling pathways by which the cells mount an appropriate defence response. Whilst TLRs are type I transmembrane proteins, NLRs are cytosolic proteins. Determination of the genome sequence of the purple sea urchin (*Strongylocentrotus purpuratus*) revealed this echinoderm had a greatly expanded repertoire of TLR/NLR innate immunity genes in comparison with vertebrates possessing an acquired immune system [1]. The open reading frame (ORF) of a number of NLR genes in *S. purpuratus* (and organisms from other phyla) commence with a leader sequence with sequence similarity to ‘2A/2A-like’ oligopeptide sequences found in many virus genomes, which mediate an unusual translational ‘recoding’ event in which translation arrests at the C-terminus of 2A (mid-ORF; no stop codon present), but then may recommence the synthesis of the peptide sequences possessing an acquired immune system [1]. The open reading frame (ORF) of a number of NLR genes in *S. purpuratus* (and organisms from other phyla) commence with a leader sequence with sequence similarity to ‘2A/2A-like’ oligopeptide sequences found in many virus genomes, which mediate an unusual translational ‘recoding’ event in which translation arrests at the C-terminus of 2A (mid-ORF; no stop codon present), but then may recommence the synthesis of the peptide sequences possessing an acquired immune system [1].

Many 2As possess only partial recoding activity: if, for example, a particular 2A sequence recodes translation only 50% of the time, then in 50% of the translation products the peptide bond is formed and the resulting translation profile is a mixture of the recoded (‘cleaved’) products (50%) plus the fusion protein (50%).

**Cellular 2A-like Sequences**

Database probing with a motif characteristic of 2A (-D(V/I)ExNPNP-) revealed the presence of ‘2A-like’ sequences in the genomes of many other RNA viruses but, surprisingly, also multiple occurrences within the genome of *S. purpuratus*. Here, 2A-like...
sequences were observed within two main types of cellular sequence: non-LTR retrotransposons [7,8] and certain NLRs [6]. In the latter case our surprise was compounded in that these particular 2A-like sequences formed the N-termini of certain NLR proteins; until that point all of the (virus) 2A/2A-like sequences we had studied were internal features used for the co-expression of protein domains up- and down-stream of 2A (Figure 1). These cellular NLR-2As were of approximately the same length as virus 2As (~25-30aa), but comprised a higher proportion of hydrophobic residues and were predominantly flanked by charged residues – suggestive of a signal sequence and, indeed, bioinformatic analyses confirmed these NLR-2As could be signal sequences. But how to show these NLR-2As could (i) mediate translational recoding and (ii) could function as N-terminal signal sequences? Given the lack of immortalised S. purpuratus cell-lines, the limited availability of primary cell-lines, the lack of anti-NLR antibody probes etc., we chose to recapitulate the system using fluorescent reporter proteins (to study the effects upon protein localisation) and, since the function of N-terminal sequences is conserved across kingdoms, we used both immortalised, readily transfectable, mammalian (HeLa) cells and plant (Nicotiana benthamiana) leaf epidermal cells. Our data showed that these NLR-2As could both mediate (partial) translational recoding and act as N-terminal signal sequences [6]. When the NLR-2A recoded itself from the fluorescent reporter protein (mCherryFP), this protein localised to the cytoplasm. In the proportion of translation products where the NLR-2A did not recode – remained as an N-terminal feature of mCherryFP - then mCherryFP was secreted from the cell. Our data showed that in mammalian cells the NLR-2A was removed from the secreted mCherryFP, presumably by signal peptide peptidase.

**Innate Immune Signalling in *S. purpuratus***

The systems outlined above allowed us to demonstrate that these NLR-2As were a new type of bi-functional translational recoding/signal sequence – but what are the implications for cell signalling in the innate immune system of *S. purpuratus*? TLRs are type I transmembrane proteins. Upon activation by PAMPs/DAMPs binding to the leucine-rich repeat domain (LRR), TLRs recruit adapter proteins within the cytosol of the immune cell in order to initiate the propagation of signal transduction pathways (Figure 2, Panel A). NLRs are, however, entirely cytosolic proteins detecting and signalling the presence of PAMPs/DAMPs present within the cytoplasm (Figure 2, Panel B). Recognition of the ligand by the LRR leads to the NOD/NBD – nucleotide-binding (NACHT) domain (Panel B). The presence of a functional N-terminal signal sequence (functional at least in mammalian and plant cells) together with putative transmembrane/internal signal-anchor/stop-transfer domains between the NACHT and LRR domains raises a number of questions with regards the sub-cellular localisation, potential membrane association (and, if this is the case, the protein orientation in the membrane) of these *S. purpuratus* NLR proteins. As shown in (Figure 3) (left panels), if the 2A-like signal sequence does not mediate a translational recoding event (remaining fused to the NLR), then the protein could either be secreted from the cell (no effect of putative TMD/internal signal-anchor/stop-transfer sequences), or, become a type 1 transmembrane protein (putative TMD/internal signal-anchor/stop-transfer sequence functional), although it is difficult to envisage a signalling function of such a molecule either being completely secreted from the cell or with this particular orientation within the membrane. As shown in (Figure 3) (right panels) shows the possibilities if the 2A-like signal sequence does mediate a translational recoding event (2A-like signal sequence not present at the N-terminus of the NLR): the NLR could become cytoplasmic (no effect of putative TMD/internal signal-anchor/stop-transfer sequences), or, perhaps, become either a type I or a type II transmembrane protein (internal signal-anchor/stop-transfer sequence functional). The orientation of the protein in the membrane is determined by the difference in the charges of the 15 residues flanking the first internal signal-anchor/stop-transfer sequence, with the more positive portion facing the cytosol [10].
Our paper presented analyses of the function of these N-terminal 2A-like signal sequences of NLR genes in *S. purpuratus* [6]. It should be noted, however, that we have detected a similar 2A-NLR gene organisation in other phyla; the cephalochordate *Branchiostoma floridae*, the sponge *Amphimedon queenslandica*, the mollusc *Lottia gigantea* and the arthropod *Ixodes scapularis* [11].

These data, plus subsequent bioinformatic analyses presented here, raise a number of important questions as to the function of these 'NLR-like' genes in these organisms. It may prove to be the case that they may have not only expanded the number of innate immunity genes, but also expanded the functionality of some of these NLR proteins – potentially involving novel signalling pathways.

**Acknowledgement**

The authors gratefully acknowledge the support of the UK Biotechnology and Biological Sciences Research Council (BBSRC) for the work on cellular 2A-like sequences.

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