From the archive

The use of symbols in chemistry, and excitement at an aquarium over an octopus baby boom.

100 years ago

Symbols are both an aid and an obstacle to thought ... There is always the danger ... faced by the student of chemical science, for without symbols systematic advance is impossible: the symbols are based on a theory and permit the representation of that theory in detail ... The first symbols ... for the metals known to the ancients, indicated ... their supposed association with the planets and the gods ruling them. Thus the solar disk stood for gold, the lunar crescent for silver ... and so on. Towards the end of the eighteenth century we see the beginnings of our present system of elementary symbols ... [A] system ... of formulation in use at the present time for the representation of elements and the composition of compounds ... is never likely to be superseded ... [W]hether the symbols we use are simple or complicated, we should always be clear as to their true significance, and be on our guard against their distracting our thoughts from the realities which they partly reveal and partly obscure.

From *Nature* 30 June 1923

150 years ago

The fine specimen of the Octopus brought to the Brighton Aquarium from the French Coast ... and suspected at the time ... to be a female, has just verified this anticipation by depositing numerous eggs ... within a few inches of the front glass of its tank; thus affording every facility ... to watch their progress towards maturity from day to day. The eggs were deposited on Thursday last ... since which time the parent has vigilantly guarded them, usually encircling and partly concealing the whole within a coil of one or more of her snake-like arms ... The mate of the interesting parent is a fine fellow brought from the Cornish Coast last February. On the arrival of his fair companion he immediately vacated his oyster grotto in her favour and for many subsequent days lavished upon her the most assiduous attention.

From *Nature* 26 June 1873

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Cell death

The role of NINJ1 protein in programmed destruction

James C. Whisstock & Ruby H. P. Law

The protein NINJ1 drives membrane rupture associated with certain types of cell death. Investigation of NINJ1 reveals mechanistic details of how it functions, raising the possibility of developing new therapeutics. **See p.1065 & p.1072**

Programmed cell death (PCD) is a central process for the removal of damaged or unwanted cells in the context of cancer and development. It also has a key role in the normal functioning of the immune system[1]. In the realm of immune-system defences, PCD can rob a bacterium or virus of its host-cell home and thereby halt the spread of infection[1]. The control of death is so important in life that at least three complex pathways for PCD – termed apoptosis, pyroptosis and necroptosis – have evolved[1]. Writing in *Nature*, Degen *et al.* (page 1065)[2] and Kayagaki *et al.* (page 1072)[3] shed light on how the protein NINJ1 functions in the final stages of certain types of PCD.

PCD and the mechanisms behind genetically encoded death have long fascinated biologists. The most-studied form of PCD, originally observed and reported by Karl Vogt in 1842 and now termed apoptosis, generally ends 'quietly' with the dying cell shrivelling up and fragmenting before being ingested by macrophage cells, which handle such garbage disposal. By contrast, both pyroptosis and necroptosis end with a bang – the plasma membrane that surrounds the cell and is crucial for cellular integrity ruptures and the cell contents leak out[4]. In certain circumstances, apoptotic cells can progress to a secondary phase (called secondary necrosis of apoptosis), in which the dying cells develop 'bubble-like' structures and the plasma membrane eventually ruptures[1].

If cells rupture in this fashion and their cytoplasmic contents spill out, proteins such as lactate dehydrogenase (LDH) and large pro-inflammatory molecules, termed damage-associated molecular patterns (DAMPs), which are normally contained in the cellular cytoplasm, escape from the cell (Fig. 1). The presence of DAMPs outside cells indicates to the immune system that all is not well, and a sustained and sometimes extremely intense inflammatory response develops to counter the threat[1,4]. Pyroptosis, necroptosis and plasma-membrane rupture in apoptosis can all be triggered in response to infection by viruses or bacteria, and all underlie tissue damage in many chronic conditions, including cancers and neurodegenerative diseases[1,4].

Central to pyroptosis is the pore-forming protein GSDMD (refs 5, 6). This normally exists in soluble form in the cytoplasm, but when activated during pyroptosis, it assembles to form pores in the plasma membrane. These pores are quite small (around 21 nanometres in diameter) – large enough to let pro-inflammatory molecules escape the dying cell but too small to permit the release of large DAMPs and LDH. For a long time, GSDMD pores were thought to promote a passive process of cell rupture through the non-selective flux of ions and the induction of osmotic stress. However, this view was overturned by a study[7] demonstrating that plasma-membrane rupture and the release of LDH and DAMPs in pyroptosis require NINJ1. As that study and the work by Kayagaki *et al.* show, NINJ1 is also needed for plasma-membrane rupture in

apoptosis, an event that occurs after cell death has begun and that is independent of GSDMD.

NINJ1 is a small transmembrane protein that comprises an extracellular amino-terminal region and two carboxy-terminal transmembrane helices. The protein was previously identified as an adhesion molecule that is produced after nerve injury[8]. Accordingly, its identification as a key mediator of membrane rupture in cell death[7] was a major and unexpected finding.

The precise details of NINJ1's role in membrane rupture and the molecular mechanism underlying NINJ1 function were not fully understood. Degen and colleagues address this issue, and show through the use of various imaging techniques and biochemical approaches that NINJ1 can undergo a conformational rearrangement that results in the self-association of multiple copies of the protein (oligomerization). This structure penetrates the membrane, creating large, irregularly shaped holes.

Kayagaki *et al.* report their development of a potent anti-NINJ1 antibody called clone D1, which blocks NINJ1 oligomerization and prevents cell rupture in the context of pyroptosis and apoptosis. Through cellular studies and by examining mouse models of cell death associated with hepatitis, Kayagaki and colleagues show that either genetic deletion of the gene encoding NINJ1, or NINJ1 inhibition mediated by clone D1, lessened plasma-membrane rupture and reduced the release of LDH and DAMPs compared with the case for cells with functional NINJ1.

Degen *et al.* used a crosslinking technique to reveal that NINJ1 oligomerization takes place after GSDMD pores form, and that LDH release occurs as NINJ1 oligomers form. Generating a functionally active fluorescent form of NINJ1 enabled the authors to observe this protein clustering on the plasma membrane during pyroptosis. Building on this, Degen *et al.* used super-resolution microscopy to reveal that NINJ1 clusters as long, highly branched filaments and large, ring-shaped structures in the membrane. Remarkably, some of these filaments reach micrometre lengths.

Using a combination of size-exclusion chromatography, live-cell imaging and negative-stain electron microscopy, Kayagaki and colleagues showed that NINJ1 oligomerizes into large filaments and that the assembly of these structures is blocked by clone D1. Crucially, clone D1 prevented NINJ1-mediated release of a cargo, which was encapsulated in lipid (a liposome), indicating that the antibody was functional in the context of a model-membrane system.

Degen and colleagues produced NINJ1 *in vitro*, and used a detergent to extract an oligomeric form that they analysed using single-particle cryo-electron microscopy (cryo-EM). The structure obtained reaches a near-atomic level of resolution, and reveals that the basic repeating unit in a NINJ1 filament comprises four α-helical structures (Fig. 1). Two hydrophobic transmembrane α-helices in the C terminus (α3 and α4) stack together to form the core of the filament, and one of the two helices in the N-terminal region (α2) packs against α3 and α4. The second of the two helices (α1) sits at a distinctive angled orientation to α2 and forms extensive intra-subunit contacts that link adjacent NINJ1 proteins.

The authors found that the molecular arrangement of α2, α3 and α4 is consistent with the model generated using the protein-structure prediction tool Alphafold[9]. By contrast, and unsurprisingly, the crucial role of α1 and the molecular interactions that this helix makes in driving NINJ1 self-assembly were not predicted by Alphafold. This reinforces the necessity of using experimental methods in structural biology, because predictive techniques could not have revealed either the remarkable function of NINJ1 or the mechanistic complexity of this relatively small protein.

Degen *et al.* provide an explanation for how NINJ1 ruptures the membrane. A key observation is that α1 and α2 have one face that is hydrophobic, with the other being hydrophilic. Accordingly, in live cells, in which NINJ1 has not oligomerized, it has been proposed that α1 and α2 are in the extracellular environment, not in the membrane. However, during cell death, α1 and α2 insert into the membrane and thereby drive NINJ1 oligomerization mainly through interactions between α3 and α4 and the hydrophobic face of α1 from an adjacent subunit. Such structures would disrupt membrane integrity and form a lesion, or pore, through the introduction of the hydrophilic faces of α1 and α2 into the hydrophobic membrane. Degen *et al.* support these mechanistic ideas through comprehensive analysis of NINJ1 mutants and molecular-modelling studies.

The region of NINJ1 to which clone D binds was mapped by Kayagaki and colleagues as being at the end of NINJ1's C terminus – a flexible region that is not captured in the cryo-EM structures. Previously, the N and C termini have been shown (through mutational studies) not to be required for rupture of the plasma membrane[7]. The authors suggest that clone D1 functions to prevent NINJ1 oligomerization by blocking NINJ1 proteins from self-associating.

The two studies reveal how NINJ1 comes into play in the final act of PCD, ensuring total disruption of the cell membrane and release of DAMPs from the cytoplasm. NINJ1 thus joins a crowded field of pore-forming proteins involved in PCD. Such molecules have central and multiple roles in apoptosis (as is the case for perforin)[10], pyroptosis (GSDMD)[5,6] and necroptosis (for example, bacterial pore-forming toxins or the protein MLKL)[11,12].

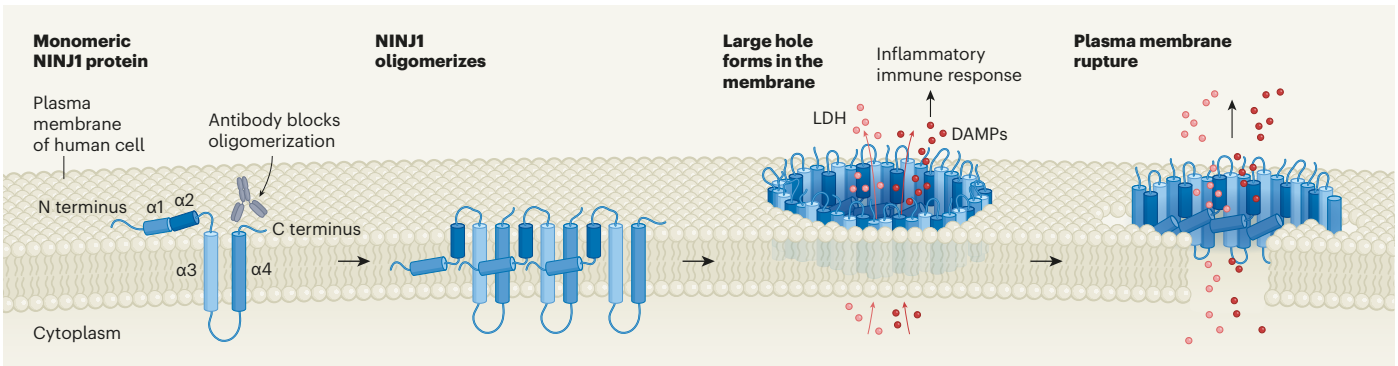

Figure 1 | How the NINJ1 protein acts in cell death. When cells die by a process called pyroptosis, or by certain forms of cell death termed apoptosis, NINJ1 mediates rupture of the plasma membrane. A single (monomeric) NINJ1 protein contains four α-helices termed α1, α2, α3 and α4 between its amino (N) and carboxy (C) termini. Kayagaki *et al.*[3] report the development of an antibody that prevents NINJ1 proteins from assembling in the oligomerization process that precedes cell death. Degen *et al.*[2] present structural data for the changes that occur when NINJ1 mediates cell death. Before NINJ1 is activated, α1 and α2 lie outside the cell. As oligomerization occurs, the orientations of the helices change, and α2 enters the membrane such that it is positioned against α3 and α4. Contacts between individual proteins are mediated by α1, which has a distinctive angled orientation. The formation of a large pore of oligomerized NINJ1 enables proteins such as lactate dehydrogenase (LDH) and inflammatory molecules called DAMPs to leave the cell. The presence of DAMPs outside the cell triggers an immune response. The plasma membrane eventually ruptures. (Adapted from Fig. 4 of ref. 2.)

Many questions remain. For example, how are the different types of PCD integrated and controlled to achieve optimal release of DAMPs? What precisely triggers NINJ1-induced pore formation? Finally, the identification of antibodies that can function to inhibit NINJ1's pore-forming activity *in vivo* raises the intriguing question of whether NINJ1 might represent a therapeutic target for certain chronic inflammatory diseases.

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Atmospheric chemistry

Halogen-containing gases cool the climate

Laura Revell

Simulations using a model of the Earth system have shed light on the role of short-lived halogen-containing gases in climate change. The findings suggest that these gases should now be included in all Earth-system models. **See p.967**

Short-lived gases that contain the halogen elements chlorine, bromine or iodine are widespread in Earth's atmosphere. These compounds, which last for no longer than six months, are involved in numerous chemical processes in the lower atmosphere, and affect the abundance and lifetimes of gases and particulates that contribute to climate change. However, the collective influence of short-lived halogen-containing (SLH) compounds on the global climate has remained unknown. On page 967, Saiz-Lopez *et al.*[1] provide a comprehensive assessment of the role of these compounds in global climate change, and find that they contribute to climate cooling through their involvement in atmospheric chemistry.

Nearly five decades ago, research into halogen-containing gases was sparked by the realization that they posed a threat to Earth's protective ozone layer[2]. Of particular concern were chlorofluorocarbons (CFCs), which are inert in the lower atmosphere, but deplete ozone in the stratosphere, around 10–50 kilometres above Earth's surface. In 1987, the Montreal Protocol on Substances that Deplete the Ozone Layer was implemented to phase out the use of CFCs and protect the ozone layer. However, it has since become apparent that some SLH compounds also reduce the levels of ozone in the stratosphere[3,4], whereas others have crucial roles in the chemistry of the lowermost atmosphere[5] (the troposphere).

Understanding the behaviour of halogen-containing compounds in the troposphere has been a challenge. Short-lived compounds are emitted by a wide array of sources, both natural (such as the ocean, polar ice and volcanic plumes[5]) and human-induced[3]. Their brief lifetimes, ranging from seconds to months, mean that it is difficult to measure atmospheric concentrations accurately[6].

Nevertheless, over the past few decades, scientists have come to realize that SLH compounds are ubiquitous in the troposphere, and contribute to climate change by warming and cooling the atmosphere (Fig. 1). The cooling occurs through their ability to destroy ozone, which heats the stratosphere by absorbing sunlight and acts as a greenhouse gas in the troposphere[6]. Warming happens when SLH compounds slow the formation of atmospheric aerosols, which cause cooling at ground level by reflecting sunlight back to space[7]. Furthermore, SLH compounds decrease the abundance of the hydroxyl radical[5] – a chemical species known as the detergent of the atmosphere, because it efficiently removes pollutants from the air. Reduced levels of hydroxyl radicals mean that the greenhouse gas methane is removed less rapidly from the atmosphere, resulting in a small amount of atmospheric warming.

Assessing the overall impact of the multitude of chemical reactions involving SLH compounds and their various climate feedbacks is challenging, especially considering that atmospheric composition and climate are changing rapidly. In their study, Saiz-Lopez *et al.* used an Earth-system model – a type of simulation used to inform assessments such as those made by the Intergovernmental Panel on Climate Change (IPCC) – to quantify how

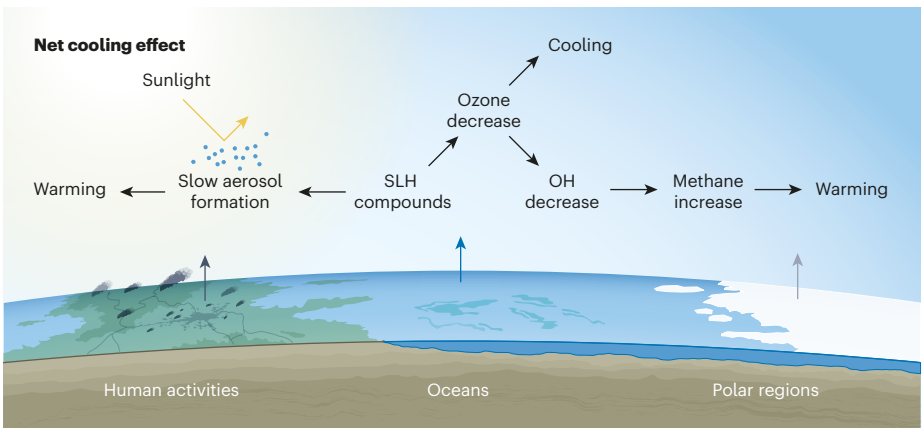

Figure 1 | How short-lived halogen-containing compounds affect climate. Gases that contain the halogen elements chlorine, bromine or iodine are produced naturally (for example, from the ocean surface and polar ice) and from human activities (such as industry and biomass burning). Saiz-Lopez *et al.*[1] analysed the pathways through which short-lived halogen-containing (SLH) compounds influence the global climate. The biggest effect is climate cooling, which occurs because SLH compounds deplete ozone (a gas that causes atmospheric warming). This cooling is partly offset by warming through two pathways: SLH compounds slow the formation of atmospheric aerosols (which cool the climate by reflecting sunlight back to space); and they decrease the levels of hydroxyl radicals (OH) in the atmosphere, which causes an increase in the abundance of the greenhouse gas methane. However, the net cooling effect is not sufficient to counteract global warming caused by carbon dioxide emissions.