As a toxin dies a prion comes to life: A tentative natural history of the [Het-s] prion

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A variety of signaling pathways, in particular with roles in cell fate and host defense, operate by a prion-like mechanism consisting in the formation of open-ended oligomeric signaling complexes termed signalosomes. This mechanism emerges as a novel paradigm in signal transduction. Among the proteins forming such signaling complexes are the Nod-like receptors (NLR), involved in innate immunity. It now appears that the [Het-s] fungal prion derives from such a cell-fate defining signaling system controlled by a fungal NLR. What was once considered as an isolated oddity turns out to be related to a conserved and widespread signaling mechanism. Herein, we recall the relation of the [Het-s] prion to the signal transduction pathway controlled by the NWD2 Nod-like receptor, leading to activation of the HET-S pore-forming cell death execution protein. We explicit an evolutionary scenario in which formation of the [Het-s] prion is the result of an exaptation process or how a loss-of-function mutation in a pore-forming cell death execution protein (HET-S) has given birth to a functional prion ([Het-s]).

KEYWORDS. [Het-s], nod-like receptors, exaptation, amyloid, incompatibility, cell death

“From the heart of all matter
Comes the anguished cry
Wake, wake, great Siva,
Our body grows weary
Of its law-fixed path,
Give us new form
Sing our destruction,
That we gain new life...”
Rabindranath Tagore

INTRODUCTION

In 1952, the geneticist Georges Rizet described, in the fungus Podospora anserina, an infectious cytoplasmic element he termed s (later renamed [Het-s]) \(^1,2\). This element is involved in a non-self recognition process known as heterokaryon incompatibility, very common in filamentous fungi and which leads to the death of the cells resulting from the...
fusion of genetically unlike strains. Incompatibility is thought off as a mechanism to protect individuality. Incompatibility systems might have evolved to prevent conspecific parasitism (exploitation of an individual by another individual belonging to the same species) and/or to limit cytoplasmic transmission of infectious elements (such as deleterious plasmids and viruses) between strains. Incompatibility is controlled by multi-allelic loci termed heterokaryon incompatibility loci (het genes). One of the incompatibility loci of Podospora anserina exists as 2 incompatible genotypes, het-s and het-S. Strains of the het-s genotype have unorthodox genetic properties, they can display 2 alternate phenotypic states termed [Het-s] and [Het-s*] that are defined by their reactivity toward het-S strains. Only [Het-s] strains are incompatible with het-S while [Het-s*] strains are neutral and compatible both with [Het-s] and het-S. [Het-s] strains contain an infectious cytoplasmic element responsible for incompatibility with het-S and upon contact with [Het-s] strains, a [Het-s*] strain is systematically converted to the [Het-s] phenotype. The cytoplasmic element appears spontaneously at a low frequency and can be eliminated in sexual crosses between [Het-s] and het-S strains. This cytoplasmic element is the prion form of the protein encoded by the het-s allele. The HET-s and HET-S allelic variants differ by 13 amino acid residues. Both are 2 domain proteins with an N-terminal folded α-helical domain termed HeLo and a C-terminal natively unfolded domain. Prion conversion of HET-S corresponds to folding of the C-terminal region into a specific amyloid β-solenoid fold. The prion forming domain contains 21 amino acid pseudo-repeats. Each repeat forms one rung of a 2-layer β-solenoid structure and comprises 4 β-strands. In each repeat, the 3 first β-strands delimit a triangular hydrophobic core while the fourth protrudes somewhat from the core. When [Het-s] interacts with HET-S, the PFD region of HET-S is converted to the β-solenoid fold. In turn, this conversion induces a conformational change in the HeLo domain which acquires a pore-forming activity, relocates to the cell membrane and causes cell death. Note that unlike what is observed in HET-S, the β-solenoid folding of HET-s does not induce toxicity. This difference is due to a particular amino acid polymorphism between HET-S and HET-S located at position 33 in the HeLo toxicity domain. The [Het-s] prion shows a very high prevalence in natural populations, in the wild, 90% of the het-s strains harbor the [Het-s] prion. What was realized only recently is that the gene adjacent to het-S in the Podospora genome encodes a Nod-like receptor showing a region of homology to the 21 amino acid elementary HET-s repeat motif. In this protein termed NWD2, the motif is N-terminal (in position 3 to 23) and present only once as opposed to twice in the HET-S/s PFD. Nod-like receptors belong to a superfamily of ATPases termed STAND which function by ligand-induced oligomerisation. The homology and genomic clustering between Nwd2 and het-S suggested the possibility of a functional relation between the 2 gene products. It was envisioned that NWD2 might serve to activate HET-S by adopting a HET-s-related fold upon ligand recognition and by converting HET-S to the β-solenoid fold. Artificial variants of NWD2 responding to known ligands efficiently induce [Het-s] prion formation in a ligand dependent manner. This activity depends on the N-terminal HET-s-like motif and point mutations predicted to affect the β-solenoid fold affect prion inducing activity of NWD2. The NWD2 HET-s-like motif alone is sufficient to induce [Het-s] formation and is able to convert HET-S to the pore-forming state. In vitro, this 21 amino acid motif forms amyloid fibrils with prion-inducing activity and solid state NMR analyses indicate that NWD2(3–23) adopts a HET-s related β-solenoid fold. These results can be interpreted in the following model: in response to its cognate ligand, NWD2 undergoes oligomerization which allows for cooperative folding of the N-terminal motif into a β-solenoid fold, this
fold then serves as a template to induce \(\beta\)-solenoid folding of the HET-S PFD region and activation of the HeLo pore-forming domain. Recent reports have revealed that prion-like signal transduction occurs in human NLRs.\(^{20,21}\) The work by Cai and co-workers in particular provides direct experimental evidence for the similarity between the processes mediated by DD (death domain) signaling and the HET-s PFD motif as it was reported that the \(\beta\)-solenoid motifs of HET-s and NWD2 can functionally replace the Pyrin domains in ASC/NLRP3 signal transduction.\(^{20}\) These recent papers are related to an emerging trend notably nourished by the work of Hao Wu stressing that higher-order complex formation constitutes a novel paradigm for signal transduction particularly in the context of immune defense and cell fate.\(^{22–24}\) Now there is a mechanistic difference between DD mediated signaling and NWD2/HET-S signaling. In the former oligomerisation involves association of folded domains, while in the latter, oligomerization is based on cooperative formation of an amyloid fold, a fold that does not pre-exist in the resting state of the NLR. The same principle of higher-order complex mediated signal transduction can be achieved by these 2 different mechanistic means. Other signaling complexes in mammals, like the RIP1/RIP3 necrosome involve amyloid-scaffolding.\(^{25}\) Interestingly, the RHIM amyloid motif which scaffolds necrosome formation appears evolutionary related to the HET-s motif.\(^{26}\)

While some aspects of NWD2 amyloid signaling have been explored, a large number of mechanistic questions now emerge. Does HET-S detach from the NWD2 amyloid hub after conversion? Does activated HET-S maintain the \(\beta\)-solenoid fold in the pore-forming state? What is the oligomerization state of assembled NWD2? How can the oligomerization geometry of the nucleotide binding and oligomerization domain be reconciled with the constraints imposed by amyloid stacking of the HET-s-like motifs? Besides, a central biological question remains open regarding the nature of the cognate ligand inducing NWD2 activation. Like plants and animals, fungi possess a large range of NLR-encoding genes which differ in particular by the type of effector domain they harbor.\(^{27}\) The modalities of innate immunity and biotic interactions that these receptors might control are only barely beginning to be tackled.

**EVOLUTIONARY ORIGIN OF [Het-s], OR HOW A LOSS-OF-FUNCTION MAY CREATE A GAIN OF FUNCTION**

The evolution of an incompatibility system poses a conundrum, how do you evolve duality? The HET-S/[Het-s] system might illustrate a possible solution to this problem. Comparative genomics indicate that the NWD2/HET-S gene pair occurs in a large number of fungal species. In contrast [Het-s] appears so far to be limited to *Podospora anserina* and the closely related *Podospora comata* species.\(^{15,28}\) It is thus reasonable to propose that the NWD2/HET-S gene pair constitutes a cell fate controlling system that is evolutionary relatively ancient and widely conserved, a hypothesis that can be nicknamed the “HET-S-first” model. Experimental evidence indicates that several single point mutations can inactivate HET-S, deprive it for the cell death inducing activity.\(^{13,15}\) In particular, the H33P mutation, in the N-terminal region of the HeLo domain abolishes pore-forming activity. This variation is the functionally relevant polymorphism that distinguishes HET-S from HET-s. The H33P change kills pore-forming activity and at the same time allows the protein to behave as a stable prion, precisely because it is not toxic anymore. Prions are like other infectious agents, tempered pathogenicity is a condition for infectivity. We thus envision that the *het-S* gene has suffered a H33P point mutation which led to the effective inactivation of the NWD2/HET-S programmed cell death pathway. Loss of this putative immune receptor must come with some fitness cost but the high redundancy of the NLR-encoding genes in fungi in general and Podospora in particular might limit the impact of this loss-of-function.\(^{27}\) One interesting observation is that loss of HET-S activity and NWD2 activity go hand in hand. In all *het-s* strains, the *nwd2* gene is in an inactivated...
pseudo-gene form. One possible explanation is that in the absence of a functional HET-S effector, NWD2 becomes useless. Effectively from this point on, duality emerged. Prion conversion of HET-s (envisioned as a loss-of-function mutant of HET-S) gives rise a novel phenotype leading to the gain of the incompatibility with the original het-S genotype. Experimentally it has been shown that het-S/het-s incompatibility participates in the limitation of the transmission of a particular infectious element (a senescence plasmid) between strains. Theory predicts that if a fitness advantage is associated with the incompatibility function then, due to frequency dependent balancing selection with advantage to the rare genotype, het-S and het-s alleles should reach equilibrated frequencies. In practice, instead of the expected 50% for each allelic type, it was found that het-s alleles are more frequent than het-S in wild populations as they are present in over 64% of the strains. One has to consider another evolutionary force to explain the success of the het-s (mutant allele) in wild populations. The het-s gene is a meiotic drive element that is able to skew Mendelian segregation in its favor in het-S x het-s sexual crosses.

In the scenario drawn here, [Het-s] is an exaptation. The term exaptation was introduced by Stephen J. Gould in 1982 to describe an evolutionary situation in which a biological structure (be it a gene, a protein, an organ, a behavioral trait...) with a given function was further co-opted to perform a different function which was not originally the target of natural selection. Since its introduction, the term has experienced a mixed career. The word drifted to the field of technology, cultural evolution and its usefulness in biology has been criticized. Yet, the use of the term, in a number of recent high-profile publications on evolution illustrates its lasting potency. We feel that [Het-s] is an interesting example of exaptation in which the process can be understood at the molecular level and pinned down to a single point mutation. In addition, the [Het-s] case is characterized by the coincidence of loss-of-function and neo-functionalization (neo-functionalization here corresponds to acquisition of the incompatibility function which confers a benefit in restricting various forms of parasitism). There is no temporal overlap between the original and neofunction. Genetically, mutational death of the gene encoding the HET-S executioner signs birth of the gene encoding the [Het-s] functional prion.

Explicitly or implicitly, yeast prions have also been regarded as exaptations. In Sup35, the unstructured NM region participates in the protein’s function in translational termination. Genetic evolution of this region might have rendered the protein competent for prionization and thus entailed the gene product with a neofunction at the population level as bet hedging device. Yet genetically, original and neo-function still co-exist. In the prionization process, loss-of-function (at the protein level) coincides with neo-functionalization (at the population level). In contrast in HET-S, in the scenario proposed here, loss-of-function occurs genetically and is irreversible. Emergence of the neofunction occurs after genetic death. Metaphorically speaking, upon prion formation, Sup35 undergoes a metamorphosis while HET-s experiences a resurrection.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

No potential conflicts of interest were disclosed.

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