Vredenburg and colleagues[4] and Briggs *et al.*[5] carried out long-term, large-scale monitoring and sampling of amphibian populations in the Sierra Nevada in California, focusing on yellow-legged frogs — *Rana muscosa* and *Rana sierrae* — the populations of which have declined in recent decades. Previous studies focused exclusively on the prevalence of infection (that is, the proportion of infected hosts), ignoring the role of infection intensity (the amount of infection per individual host) in controlling host-population losses. Instead of simply cataloguing the presence or absence of *B. dendrobatidis* and its spread among host populations, these investigators[4,5] identify a 'lethal threshold' of pathogen infection intensity, which may be the key to understanding how *B. dendrobatidis* epidemics can be controlled.

Vredenburg *et al.*[4] carried out intensive sampling of 88 frog populations over 9–13 years. Among the lakes they studied, they found that, within three years of its arrival, *B. dendrobatidis* had spread in a wave-like pattern — that is, the area covered by the pathogen increased steadily in size over time — until nearly all of the frog populations at the lake were infected. The amphibian populations did not, however, collapse until a lethal threshold of about 10,000 zoospores of the fungus per frog was reached.

The existence of such an intensity threshold may help to explain how *B. dendrobatidis* causes almost complete losses of amphibian hosts. Because of this threshold, there is a time lag between exposure and mortality, so the pathogen can spread through much of the amphibian population before disease-driven reductions in host density negatively affect the transmission of *B. dendrobatidis*. Consequently, the pathogen can cause the loss and extinction of its host population, unlike the many other pathogens that disappear as their hosts decline in numbers.

Briggs *et al.*[5] combine long-term field data with modelling analysis to investigate how some amphibian populations persist even though *B. dendrobatidis* is present in their habitat. The authors' intensive data — involving marking the animals and later recapturing them — show that, in populations that survive, infected yellow-legged frogs have fungal loads well below the lethal intensity threshold, and that these frogs have cleared fungal infection and become reinfected over the course of years, with no effect on their survival.

Previous studies suggested that genetic changes that alter host tolerance of the pathogen or pathogen virulence might explain how some amphibian populations persist in the presence of *B. dendrobatidis*. Briggs and colleagues' modelling efforts, however, hint that simple decreases in host density and the resultant reduction in pathogen transmission could account for such an outcome. This is particularly true when there are environmental reservoirs of *B. dendrobatidis*, including amphibian species or life stages (such as tadpoles) that can persist with the infection for long periods and spread it to more sensitive hosts.

This modelling work[5], which was based on a variety of biological scenarios, offers insight into both the epidemic and endemic aspects of *B. dendrobatidis* dynamics. For instance, the study predicts that infection intensity builds up rapidly when frog populations are dense, as well as under conditions that promote reinfection. If *B. dendrobatidis* reaches its intensity threshold, the infected amphibian population can become extinct. By contrast, if some members of the host population survive, then a new endemic state develops, with persistent infection in the remaining frogs.

Intriguingly, both studies[4,5] indicate that the traditional dichotomous classification of pathogens as either microparasites or macroparasites may be overly simplistic, as the dynamics of infection with *B. dendrobatidis* — a microparasite — strongly depend on infection intensity (which is usually considered only for macroparasites). This finding suggests that incorporating infection intensity into other microparasite disease models could provide insight into other host–pathogen systems.

The new papers[4,5] markedly increase the understanding of a disease that affects many amphibian populations. In particular, the types of data presented — based on long-term, extensive monitoring that generates detailed records — are largely unprecedented for analyses of many wildlife disease systems.

Nevertheless, large gaps remain in the knowledge of *B. dendrobatidis* and in how the dynamics of chytridiomycosis vary between geographical regions. The populations that these researchers[4,5] studied are from montane ecosystems that have low species diversity and relatively harsh winter conditions. Will the reported dynamics for *B. dendrobatidis* in this system explain the spread of this pathogen in, for example, lowland regions of Europe or in the tropics, where host-species density is substantially higher?

Moreover, it is still not clear precisely which vectors spread the infection, in which systems it is endemic and in which ones it is epidemic, and whether environmental changes can trigger the emergence of this pathogen. By focusing on infection intensity and the differences between epidemic and endemic states of *B. dendrobatidis* infection, Vredenburg *et al.* and Briggs *et al.* lay a valuable foundation for addressing questions such as how the intensity threshold of *B. dendrobatidis* varies across species or with environmental conditions, and what part is played by environmental cofactors such as climate change[8] in affecting the dynamics of endemic infection.

How can this information be applied so as to slow, or even prevent, population declines? As the authors of both papers propose, interventions designed to prevent *B. dendrobatidis* infection from reaching the lethal-intensity threshold could reduce extinction events. Because it is unlikely that the pathogen will be completely eradicated, the only realistic option may be to manage sensitive amphibian populations in such a way as to create an endemic state of infection. For instance, as described in a News Feature in these pages last week[9], reducing the density of susceptible frogs by capturing them before the infection wave, or by treating a subset of individuals with an antifungal agent, could reduce transmission of *B. dendrobatidis* and prevent infection intensities from becoming lethal. ■

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STRUCTURAL BIOLOGY

Immunity takes a heavy Toll

Steven A. Wasserman

Toll receptors trigger immune responses through adaptor proteins and kinase enzymes. Structural studies reveal that hierarchical assembly of these proteins into a helical tower initiates downstream signalling events.

Communication within cells often involves a series of molecular handshakes, each protein contacting the next and modifying its activity. An accessory protein may serve as a matchmaker, holding components together for the exchange of information. Until now, this model fitted well with what was known about signalling in mammalian Toll pathways, which activate innate immune defences[1]. Three proteins — MyD88 and two members of the IRAK

protein family — help to link pathogen recognition by transmembrane Toll receptors to the transcription of genes involved in defence. MyD88 and the IRAKs each contain a single death domain, a region that allows their interaction with the death domain of other proteins. But as Wu and colleagues (Lin *et al.*[2]) report on page 885 of this issue, the death domains do not form a simple chain. Rather, their X-ray crystal structure reveals that the death domains from several copies of each protein combine into a signalling tower more than 100 ångströms high.

Death domains were discovered and so named for their role in apoptosis, a form of programmed cell death. They were later found in components of signalling pathways governing immunity and inflammation. Disruption of signalling pathways by these domains contributes to cancer and a wide range of inflammatory disorders[3].

Built from six α-helices, death domains have three noteworthy characteristics[4]. First, their interaction is homotypic — that is, death domains bind only to other death domains. Second, this interaction can take three forms (I–III) that differ at their contact sites and that each lacks mirror-image symmetry. And third, the amino-acid sequence of these domains is highly variable, with the sequences of distantly related death domains differing by 90%.

The structure that Lin *et al.*[2] solve is the first to contain death domains from three different proteins. Here, the death domains of six MyD88 molecules comprise the first two of four layers in the complex. Four IRAK4 death domains form the next layer, and four domains from IRAK2 complete the structure (Fig. 1). Because the layers form at a slight tilt, the final shape is a left-handed helical tower. Type I and II interactions are found exclusively between layers, whereas type III interactions connect adjacent death domains along the spiral. The net effect is a cylindrical honeycomb with hexagonally arrayed protein–protein contacts.

How could an infection trigger assembly of this death-domain complex? Each cell-surface Toll protein acts as a sentinel for invading viruses and microorganisms: its extracellular portion binds to molecules found in microbes but not in animal cells, such as cell-wall components. The intracellular portion of these receptors has a TIR domain, which can interact with other TIR domains. Binding of a pathogen molecule to Toll receptors induces their dimerization by bringing together their TIR domains. The clustering of TIR domains, in turn, generates a binding site for MyD88, which also has a TIR domain. The Toll–MyD88 interaction raises the local concentration of MyD88 enough to initiate assembly of the signalling complex. Because self-association of MyD88 is quite weak, Toll activation is also required for MyD88 oligomerization and thus complex formation.

Combining structural data with the technique of site-directed mutagenesis, Lin and colleagues[2] demonstrate that recruitment of each IRAK4 molecule to the nascent complex involves multiple points of contact with other death domains. Addition of IRAK2 then follows to complete the hierarchical assembly process. But what prevents IRAK2 from joining earlier, and why does assembly stop at four layers? To answer these questions, the authors quantitated the extent to which the surfaces of each of the three death domains are complementary in charge and shape. These calculations reveal that the interactions of IRAK4 with MyD88 and with IRAK2 are sufficiently distinct to provide a fixed order for assembly.

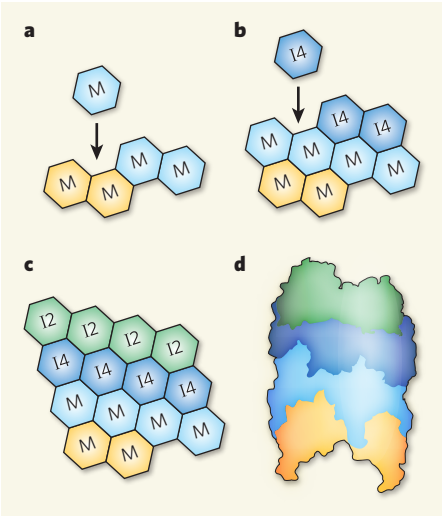

Figure 1 | Signalling-complex assembly in the Toll pathway. a–c, Assembly of the death-domain complex (viewed as if split along a roughly vertical seam and laid flat). Assembly begins when pathogen-induced dimerization of Toll recruits MyD88 molecules through TIR-domain interactions (not shown). Clustering of the MyD88 death domains (M) induces the formation of the first two layers of the signalling complex. MyD88 death domains provide composite binding sites for IRAK4 death domains (I4), which in turn recruit IRAK2 (I2). **d**, Structure of the completed signalling tower. The kinase domains of IRAK4 and IRAK2 (not shown) lie on the outside of this complex.

Lin *et al.* further show that the top and bottom surfaces of IRAK2 are incompatible with one another, preventing inclusion of extra IRAK2 molecules in the finished tower.

Once the signalling complex has formed, the IRAKs undergo phosphorylation[5]. Both IRAK4 and IRAK2 are protein kinases — phosphorylating enzymes. The immediate consequence of complex formation is IRAK4 autocatalysis. Autophosphorylated IRAK4 then phosphorylates and activates IRAK2. Activated IRAK2 leaves the complex, initiating a cascade of protein modification and destruction — through ubiquitylation, phosphorylation and proteolysis — involving other protein complexes[1]. At the end, destruction of an inhibitor protein, IκB, frees the transcription factor NF-κB to enter the nucleus and direct the expression of genes mediating immune responses.

The MyD88–IRAK4–IRAK2 complex is a key mediator of Toll signalling in humans: of the ten Toll receptors, all but one can signal through MyD88 (ref. 1). Furthermore, the activity of IRAK4 is essential for responses transduced by MyD88. IRAK4 in turn signals through IRAK2 or a closely related protein kinase, IRAK1 (ref. 5). Because the sites that contact IRAK4 are evolutionarily conserved between IRAK2 and IRAK1, Lin *et al.*[2] suggest that IRAK1 can substitute for IRAK2 in the signalling tower. This raises the question of whether IRAK1 and IRAK2, which have overlapping but distinct functions, are ever recruited into the same signalling tower.

The immune function of the Toll pathway has been conserved from fruitflies to humans[6,7]. Is the architecture of the signalling complex similarly conserved? The answer seems to be yes and no. In the fruitfly *Drosophila*, the counterparts[8] to MyD88, IRAK4 and IRAK2 are called MyD88, Tube and Pelle. From detailed structural analysis[9], interaction between the Tube and Pelle death domains matches that between IRAK4 and IRAK1 to a high degree. Furthermore, key interactions between these two sets of proteins, mapped by inactivating mutations in the fly proteins, are evolutionarily conserved. However, studies in solution indicate[10] that the death domains of MyD88, Tube and Pelle form a ternary complex rather than a higher-order assembly.

Given that a death-domain trimer suffices for Toll signalling in *Drosophila*, why does the mammalian pathway have such an elaborate architecture? Lin and co-workers suggest that a helical symmetry more readily accommodates a variable number of specific binding partners. If so, helical complexes may be found in pathways for some of the other 84 human proteins that contain the six-helical-bundle characteristic of death domains. These superfamily members participate in cell death, inflammation, DNA-damage responses, detection of intracellular pathogens and antiviral immunity[3]. Structural studies of these pathways will reveal whether other death-domain proteins also gather at their respective receptors as a flock of signals. ■

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