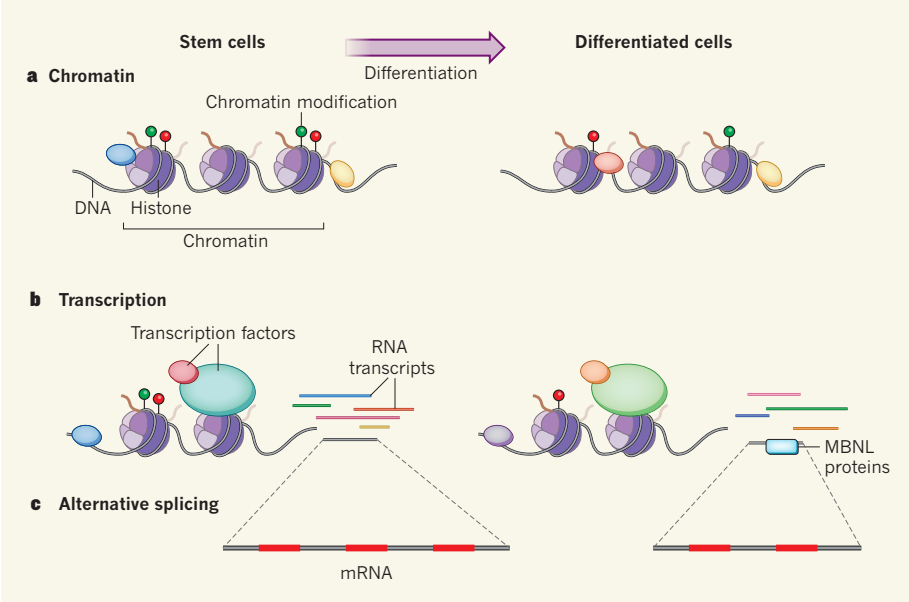


**Figure 1 | Multilayer regulation of cell differentiation.** **a**, The nature and amounts of chromatin-modifying and -remodelling proteins (blue and yellow) that bind to DNA and its associated histone proteins differ between stem cells and differentiated cells, affecting gene expression through regulation at the level of chromatin. **b**, At the level of transcription, specific transcription factors orchestrate the distinct transcript output of stem cells compared with differentiated cells. **c**, Han *et al.*[2] show that at the level of transcript processing, regulators of alternative splicing, such as MBNL proteins, govern the differences in mRNA, and thus protein, output between stem cells and differentiated cells.

cell identity? The researchers report that sites of alternative splicing in ES-cell transcripts are highly enriched in MBNL1- and MBNL2-binding motifs, and that these factors specifically bind to the sites in a unique pattern. So it seems that the binding patterns of these regulators control the omission or inclusion of protein-coding regions (exons) in the mature mRNA.

The cellular levels of MBNL proteins also seem to affect the differentiation state. Increased expression of these proteins in ES cells induced differentiation-specific alternative-splicing events, and decreased the levels of an ES-cell-specific isoform of FOXP1. Consistently, reducing expression of these proteins in differentiated cells led to a switch of the alternative-splicing program to an ES-cell-like pattern. And the efficiency of reprogramming of differentiated cells into iPS cells was greatly enhanced with reduced expression of MBNL1 and MBNL2 (the splicing pattern associated with 'stemness' was particularly prominent in cells that were successfully sustained through the later parts of the reprogramming process).

Han and co-workers' paper sets the stage for extensive follow-up studies. Understanding the exact mechanism of action of the MBNL proteins might help to identify upstream elements of this regulatory network. Moreover, the epigenetic state of ES cells — that is, genomic modifications that affect gene expression without changing the DNA sequence — is subject to continuous regulation, and a link between epigenetics and alternative splicing has been proposed[9,10]. Understanding how alternative splicing interacts with epigenetic and other networks that are known to regulate pluripotency would be fascinating. Furthermore, Han *et al.* identified many more sites of alternative splicing, and differential regulators of splicing in ES cells that they could not investigate in the current work. These should be studied, as they might provide additional insights into the mechanism by which alternative splicing controls pluripotency.

The authors' observations might also have a notable practical implication. Splicing regulators could potentially be harnessed to control the efficiency and outcome of cellular differentiation and reprogramming — akin to the use of transcription factors for these purposes. While we tune in for follow-up studies, Han and colleagues' findings will surely change the ways in which researchers examine and manipulate pluripotent cells. ■

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MALARIA

# The vector as protector

**Malaria infections are not always lethal. One reason for this may be that transmission from mosquitoes creates malaria parasites that trigger a more protective mammalian immune response.** SEE LETTER P.228

ANDREW F. READ & NICOLE MIDEO

Malaria parasites can kill people, but death is not inevitable. Most infected individuals recover, some after experiencing relatively mild symptoms or none at all. What accounts for this variability? Host factors such as the expression of sickle-cell genes or acquired immunity are part of the explanation. But it is also well known that malaria parasites themselves can be more or less nasty[1,2]. In this issue, Spence *et al.*[3] (page 228) report a set of clever experiments in a mouse model of malaria infection that shows that the conditions experienced by parasites before they reach the mammalian bloodstream can determine just how virulent they are*.

Malaria parasites transmitted to people by mosquitoes migrate to the liver, where they replicate before entering the bloodstream. For convenience, and because only blood-stage parasites cause disease, most experimental studies of malaria in humans and animals bypass the mosquito and liver stages and inject parasites directly into the bloodstream. Using the malaria parasite *Plasmodium chabaudi*, which infects rodents, Spence and colleagues compared the blood-stage infections generated by this method with those initiated naturally, by mosquito bite. They found that, compared with directly injected parasites,

*This article and the paper under discussion[3] were published online on 29 May 2013.

mosquito-transmitted parasites replicated less well once in the bloodstream and generated lower-grade infections that persisted for longer. Moreover, these parasites did not induce the severe weight loss, hypothermia and liver damage caused by parasites injected directly into the bloodstream.

Why these differences? An important clue came from the authors' finding that, in immunodeficient mice, parasites transmitted by mosquitoes grew just as well as those injected directly. This suggested that there is nothing intrinsically attenuated about parasites derived from mosquitoes. Spence *et al.* show that mosquito-transmitted parasites elicit a qualitatively different immune response in the mouse — one that better controls parasite replication and relies less on the inflammatory signalling molecules that are associated with severe disease. To try to explain this difference, Spence *et al.* conducted a genome-wide RNA analysis and found that mosquito transmission modifies the expression of about 10% of the genome of blood-stage parasites. Intriguingly, expression was most intensely regulated for gene families encoding antigenic proteins, against which the host's immune system mounts its response. The hypothesis, then, is that mosquito transmission alters subsequent antigen expression when the parasites are in the bloodstream, and that the induced gene-expression pattern elicits an immune response that more effectively contains the parasites with less collateral damage to the host.

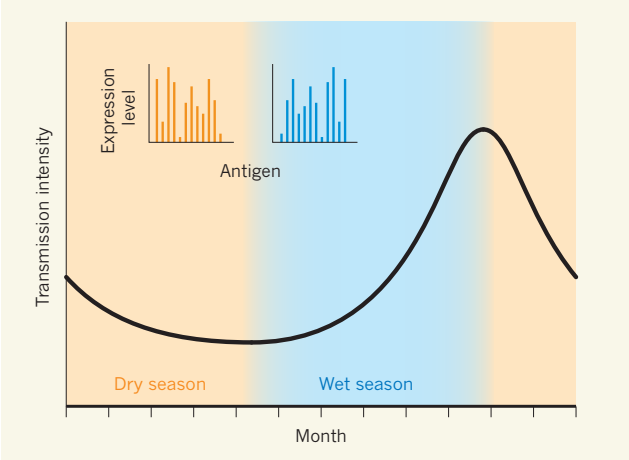


**Figure 1 | Evolutionary selection of antigenic profiles.** If variation in the antigens expressed by a parasite gives rise to qualitatively different infection dynamics and outcomes, natural selection might favour the expression of different antigenic profiles at different times or in different regions. For instance, to survive a prolonged dry season, when little to no transmission occurs, parasites with the attenuated phenotype described by Spence *et al.*[3] — causing chronic infections of low virulence — and the associated antigenic profile may be most successful. By contrast, when the rainy season begins and epidemic situations arise, parasites with antigen-expression profiles that result in rapid proliferation and transmission may be favoured. In this case, the cost of shorter infectious periods associated with rapid clearance of the parasite by the immune system, or host death, may be offset by the advantages of faster transmission to new hosts. These evolutionary forces might generate parasites that respond to cues associated with transmission (through altered gene expression) in some regions, and parasites that do not in others, such as in endemic areas where transmission occurs year-round.

It seems that it is the environment experienced by the parasites during natural transmission that triggers this 'attenuated phenotype'. That environment could be inside the mosquito itself, or it could be something experienced by the parasite in the skin soon after injection, during its journey to the liver or in the liver. Intriguingly, Spence *et al.* show that the attenuated phenotype also occurs in mice injected with blood-stage parasites isolated from other mice with mosquito-initiated infections. Thus, the phenotype is stable for several cycles of blood-stage parasite replication, although it does gradually decay over subsequent rounds of injecting these parasites into new hosts. It will be interesting to determine whether profiles of the host immune response and of parasite-antigen expression associated with attenuation decay in a similar manner.

Does this discovery mean that all future experimental malaria infections should be initiated by mosquitoes? There is no way to include mosquito transmission in *in vitro* studies of the most lethal human malaria parasite, *Plasmodium falciparum*. Immunosuppressed mice with human-cell transplants can support *P. falciparum* infections[4], but it is unclear whether the addition of one aspect of biological reality (mosquito transmission) will make up for the loss of another (the use of human parasites in mice). Mosquito infections are an option in animal models, from which much has already been learned by injecting blood-stage parasites. For example, experiments with *P. chabaudi* have shown that a powerful contributor to the severity of malaria can be the host immune response itself[5], and that competition between different parasite strains can be a potent force shaping the evolution of drug resistance[6]. The key question is not whether these phenomena still occur if more of the parasite life cycle is incorporated into the experimental work, but whether they occur in nature.

The effects of mosquito transmission on host immune response and parasite antigen expression observed by Spence and colleagues might be the independent outcomes of environmental influences, or they might be causally connected. If the latter is true, the question remains whether immunity triggers the antigenic profile or whether altered antigen expression triggers a more protective immune response. The direction of this causality could have implications for vaccine design.

In the first scenario, considering the characteristics of the immune response generated by a vaccine would be important for protecting against severe disease if vaccination does not completely block infection. In the second case, a vaccine that results in exposure to a particular antigenic profile may be crucial for developing an optimally protective response. Spence *et al.* compared infections initiated by mosquitoes and by blood-stage parasites at just one time in the blood-stage infection, but antigen expression can be highly variable in time and across host tissues[7,8], so further assessment of these profiles is needed.

If antigen-expression profiles are indeed a major determinant of malaria-parasite virulence, and if these are not completely constrained by the parasite's developmental requirements, we predict that natural selection will favour different antigen-expression profiles in different epidemiological settings (Fig. 1). If this is the case, then virulence variability due to genetic polymorphisms or phenotypic plasticity will be common in nature. This might explain apparently contrasting experimental results. For example, Spence *et al.* found that mosquito transmission attenuated parasite replication in two clones of *P. chabaudi*, but earlier experiments using a different clone found no such effect[9]. Similarly, physicians who deliberately infected people with *P. falciparum* to treat neurosyphilis reported the same clinical picture regardless of how the infection was initiated[2]. Clearly, much is yet to be learned about how malaria parasites make people sick, and about the role of the mosquito vector in modulating the disease it initiates. ■

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