These findings indicate that Foxo1 is indeed an important target for miR-182 *in vivo*. In a different model, this time using infection with lymphocytic choriomeningitis virus, they show that overexpression of miR-182 causes population expansion of the transduced helper T cells of up to 14-fold. Finally, in a model of ovalbumin-induced arthritis, they find that helper T cells loaded with the antagomir to miR-182 are unable to expand their populations, which results in much less disease severity. These *in vivo* experiments are important, as three separate models show a clear role for miR-182 in helper T cells, which indicates the central importance of miR-182 in the population expansion of helper T cells.

One compelling aspect of this study, and indeed of other studies in which miRNAs are manipulated in immunity, is the substantial immunomodulatory effect of miRNAs in complex models of immunity and inflammation. One such example is the manipulation of miR-21 in macrophages during Toll-like receptor signaling, which leads to suppression of IL-10 production[3]. This occurs via the miR-21 target PDCD4, an inhibitor of translation of IL-10. Preventing the targeting of PDCD4 mRNA by miR-21 leads to more PDCD4, which then blocks IL-10. In the study by Stittrich and colleagues, there is a blockade of miR-182 in helper T cells, which leads to more Foxo1, which then prevents helper T cell clonal expansion[2]. This in turn has an anti-inflammatory effect in a model of arthritis. However, if miR-182 is overexpressed, there is an enhanced response to lymphocytic choriomeningitis virus. One slightly puzzling aspect of this study[2], however, concerns IL-2. T cell population expansion can still occur in IL-2–deficient mice, and such mice develop an aggressive autoinflammatory disease[4]. Most studies attribute this to defective regulatory T cell function. In the *in vivo* models tested here, however, other helper T cell populations must be important, as there is an anti-inflammatory effect if miR-182 is targeted with antagomirs[2]. Presumably, these antagomirs are targeting T_H1 and T_H17 cells and interfering with their population expansion, although this is not explicitly demonstrated. As all helper T cell subtypes seem to require the miR-182 for population expansion, under what circumstances might there be selective expansion of subpopulations? Other cytokines must be able to induce miR-182, including IL-3, IL-5, IL-7, IL-9 and IL-15, as these can all activate STAT5. As the authors themselves somewhat enigmatically state, IL-7 is unable to induce miR-182. The particular response of each helper T cell subtype to the cytokine environment induced during an infection may therefore be important.

The therapeutic possibilities here are clear. If miR-182 can be boosted specifically in helper T cell populations, there could be an adjuvant effect, or if regulatory T cells are specifically targeted, there could be an anti-inflammatory effect. Equally true, if miR-182 can be interfered with via antagomirs, specifically in helper T cell populations other than regulatory T cells, there could be an antiallergic effect (in the case of T_H2 cells) or an anti-inflammatory effect (in the case of T_H1 cells and perhaps more dramatically, T_H17 cells). Furthermore, the IL-2–miR-182 axis is clearly critical for the clonal expansion of activated naive helper T cells. However, it remains unclear whether this pathway has a role in the clonal expansion of effector and/or memory cells, particularly T_H1 or T_H2 cells, which express little if any IL-2 on their own. Future work on miR-182 in helper T cells, particularly in human systems, will yield deeper understanding of the regulation of helper T cells. Therefore, there is another layer of control in helper T lymphocytes that is probably important for their functioning in health and disease.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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PYHIN proteins: center stage in DNA sensing

Delphine Goubau, Jan Rehwinkel & Caetano Reis e Sousa

Innate immune responses to pathogens are often triggered by nucleic acids, including DNA delivered to the cytoplasm of cells. IFI16 is a newly identified cytoplasmic DNA sensor that induces the transcription of genes involved in the innate response.

Infections are countered by a variety of both constitutive and inducible innate defense mechanisms that limit the replication and spread of pathogens. The inducible components of innate defense rely on innate immune sensors that detect the presence of potential pathogens and signal to initiate immunity. The recognition of nucleic acids has emerged as one of the strategies used for pathogen detection. For example, the Toll-like receptors TLR7 and TLR8 sense RNA, and TLR9 senses DNA, delivered to the endosomal compartment during virus invasion. Furthermore, the RNA helicase RIG-I–like receptors detect RNA molecules that accumulate in the cytoplasm of cells infected with various viruses[1]. Engagement of any of these receptors triggers a signaling pathway that culminates in the expression of genes involved in innate responses, including those encoding type I interferons (IFN-α/β). These interferons contribute to a heightened immune state essential for immediate virus control and coordinate cellular events that lead to induction of adaptive immunity. In this issue of *Nature Immunology*, Unterholzner *et al.* report that human IFI16 (interferon-inducible protein 16) and its closest mouse homolog, p204, are cytoplasmic DNA sensors that directly signal the induction of IFN-α/β and other proinflammatory mediators[2].

The introduction of DNA into the cytosol of cells, which is normally a DNA-free environment, induces IFN-α/β[1,3]. This can happen naturally during infection with DNA viruses (such as herpes simplex virus type 1 or vaccinia virus) or bacteria (such as *Listeria monocytogenes* or *Legionella pneumophilia*) or can be mimicked experimentally by DNA transfection. Such findings have stimulated efforts to identify the pathways that mediate sensing of DNA in the cell cytosol and link it to IFN-α/β induction. Progress in this area suggests that the detection of cytosolic DNA requires multiple and possibly redundant sensors that converge on the signaling molecule

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STING and the kinase TBK-1 and lead to activation of the transcription factor NF-κB and TBK1-mediated phosphorylation of the transcription factor IRF3 (refs. 2,3). NF-κB and IRF3 then translocate to the nucleus and induce expression of the genes encoding IFN-α/β and other genes encoding molecules involved in the innate response. DAI (also known as ZBP1 or DLM-1), as well as RNA polymerase III, have been identified as DNA sensors that are able to induce IFN-α/β via IRF3 and NF-κB. DAI binds double-stranded DNA and directly initiates signaling[4], whereas RNA polymerase III acts indirectly by transcribing AT-rich DNA into uncapped 5′ triphosphate–bearing RNA, which serves as an agonist for RIG-I (refs. 5,6). Activated RIG-I then signals through the mitochondrial adaptor MAVS (**Fig. 1**). However, the RNA polymerase III pathway cannot account for the induction of IFN-α/β mediated by non–AT-rich DNA, and DAI has been shown to be largely redundant for DNA sensing[5–9]. Therefore, the existence of at least one additional DNA sensor has been hypothesized. A recent paper has characterized LRRFIP1 as a protein that detects nucleic acids and signals via β-catenin, which acts together with IRF3 to induce transcription of the gene encoding IFN-β[10]. The work by Unterholzner *et al.* now identifies the human IFI16 protein and its mouse homolog p204 as additional sensors of cytoplasmic DNA[2].

Poxviruses such as vaccinia virus replicate in the cytoplasm and induce IFN-α/β. The DNA genome of these viruses contains multiple copies of a short conserved sequence in the terminal repeat regions. Unterholzner *et al.* use synthetic DNA corresponding to this 70–base pair motif of vaccinia virus (which they call "VACV 70mer") and find that it potently induces IFN-β when transfected into monocytic cell lines such as RAW264.7 and THP-1 but not when transfected into HEK293 human embryonic kidney cells[2]. Interestingly, published reports have demonstrated that HEK293 cells respond to DNA only via the RNA polymerase III pathway[5,6]. Consistent with the inability of HEK293 cells to respond to VACV 70mer, Unterholzner *et al.* demonstrate that the stimulatory activity of VACV 70mer in monocytic cells is independent of RNA polymerase III (ref. 2). It is also independent of DAI or TLRs but is dependent on the downstream signaling molecules STING, TBK1 and IRF3, which leads the authors to experiments aimed at identifying the putative new sensor involved. Unterholzner *et al.* use the VACV 70mer as bait to fish for DNA-binding proteins from cytosolic extracts of THP-1 cells and identify IFI16 by mass spectrometry[2]. IFI16 is part of the PYHIN protein family (pyrin and HIN200 domain–containing proteins; also known as p200 or HIN200 proteins). PYHIN proteins have been linked to cell proliferation, survival and differentiation and are characterized by at least one carboxy-terminal HIN domain and an amino-terminal pyrin domain[11]. There are three additional PYHIN family members in humans, IFIX (Pyhin1), MNDA and AIM2; each contains one HIN domain and one pyrin domain[11]. However, IFI16 is unique in that it contains two HIN domains. A search for proteins with similar domain structure shows that the closest homolog of IFI16 in mouse is p204, which shares 37% amino acid identity with IFI16.

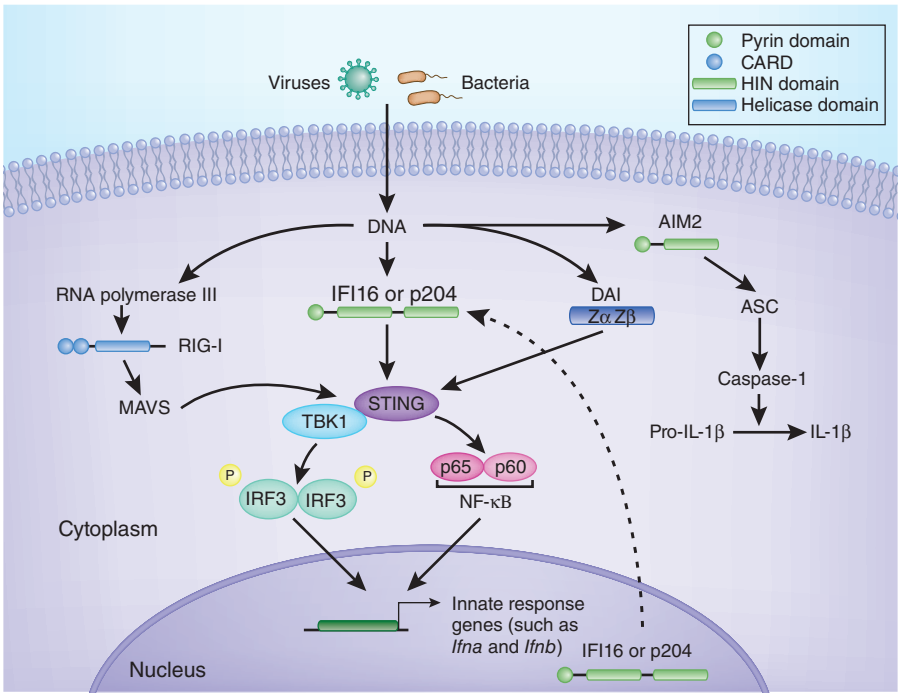

Figure 1 The PYHIN proteins IFI16 and p204 detect cytosolic DNA, which leads to the induction of type I interferon. Infection with viruses or bacteria can trigger the release of DNA into the cell cytosol, which is a DNA-free environment in healthy cells. This DNA can be sensed by AIM2, which forms an inflammasome with ASC and caspase-1 to mediate processing of the IL-1β precursor pro-IL-1β. Intracellular DNA can also trigger the transcription of genes involved in the innate response, such as those encoding IFN-α/β (*Ifna* and *Ifnb*), via various DNA receptors, including RNA polymerase III (which is selective for AT-rich DNA), DAI (which has Zα and Zβ DNA-binding domains) and IFI16 (p204). IFI16 and p204 can sense DNA via their two HIN domains. Once activated, IFI16 and p204 interact with STING and trigger TBK1-mediated phosphorylation of IRF3 and activation of NF-κB (p65 and p60 are NF-κB subunits). Those transcription factors then translocate to the nucleus and induce IFN-α/β expression. A fraction of IFI16 is found in the cytosol, yet this protein is predominantly localized in the nucleus, which suggests the possibility of active exchange between the two pools. CARD, caspase-recruitment domain.

Published work has identified another PYHIN protein, AIM2, as a mediator of innate immune responses to cytosolic DNA. AIM2 binds cytosolic double-stranded DNA through its single HIN domain and promotes the assembly of an inflammasome via pyrin domain–pyrin domain interactions with the adaptor molecule ASC[3]. The inflammasome acts as a platform for caspase-1 activation, which promotes proteolytic maturation of the proinflammatory cytokine interleukin 1β (IL-1β). AIM2, however, has no role in the induction of IFN-α/β[3]. Conversely, IFI16 does not seem to be involved in inflammasome activation or in IL-1β production[12–14]. Instead, Unterholzner *et al.* identify telltale signs indicative of a role for IFI16 and its mouse ortholog p204 in coupling DNA sensing to induction of the genes encoding IFN-α/β and other genes encoding molecules involved in the innate response[2].

Most sensors of nucleic acid involved in IFN-α/β induction are themselves interferon inducible. IFI16 mRNA is induced by exposure to IFN-α[11], and the Unterholzner *et al.* show that the expression correlates with the ability of cells to produce IFN-α/β after DNA transfection[2]. Furthermore, in response to transfection with VACV 70mer, IFI16 associates with downstream components of the cytosolic DNA signaling pathway, such as STING and TBK1. Finally, downregulation of the expression of IFI16 or p204 mediated by small interfering RNA impairs the innate immune response to cytosolic DNA. In knockdown experiments,

NF-κB and IRF3 fail to translocate to the nucleus in response to transfection of VACV 70mer DNA or infection with herpes simplex virus type 1 (a DNA virus), and induction of IFN-α/β, tumor necrosis factor, IL-6 and the chemokine CXCL10 is partially impaired.

These data demonstrate a role for a PYHIN protein in the induction of IFN-α/β and identify an additional PYHIN family member beyond AIM2 that is involved in the cell-intrinsic response to DNA. The authors propose that AIM2 and IFI16 should therefore be grouped into a new family of receptors called 'AIM2-like receptors'. Whether other PYHIN proteins are also important in DNA recognition remains an open question.

The discovery of IFI16 and p204 as cytosolic DNA sensors is as exciting as it is intriguing. The involvement of IFI16 is somewhat of a surprise, as IFI16 is predominantly nuclear[11]. Nevertheless, Unterholzner *et al.* are able to affinity-purify IFI16 from cytoplasmic extracts and show by microscopy that a small fraction of IFI16 is present in the cytoplasm, where it localizes together with transfected DNA[2]. Although this shows that IFI16 can enter the cytoplasm, it is unclear where the initial binding to DNA occurs. This is particularly interesting in the context of infection with herpes simplex virus type 1, as this virus replicates in the nucleus. It is not inconceivable that IFI16 recognizes DNA in the nucleus and then shuttles to the cytoplasm to engage the downstream signaling pathway. This raises the question of whether self DNA in the nucleus is also recognized by IFI16 or p204 or whether it is somehow shielded by structural proteins and/or chemical modifications. However, responses to self DNA could be avoided if only the cytosolic pool of IFI16 or p204 is able to recognize DNA and/or to initiate signaling. It will be interesting to determine what happens to IFI16 and p204 and their associated signaling pathways in mitotic cells at the time the nuclear envelope breaks down, when self DNA comes into contact with the cytosol.

This study provides evidence that IFI16 and p204 can sense the presence of foreign DNA and marks an important step in the understanding of cell-intrinsic innate immunity. Assessment of the wider role for IFI16 and p204 in the immune response to bacterial and viral infection awaits the generation of p204-knockout mice and the discovery of *IFI16* mutations associated with human susceptibility to infectious disease.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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