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and on both sides by a multipolar microtubule array (Figure 1C). The authors contend that in the latter case, although spindle microtubules associate with all of the chromatin, the multipolar and disorganized nature of the spindle structure would not be able to function properly to segregate chromosomes. Therefore, at a critical chromatin size, the same bipolar-to-multipolar transition that occurs for circular chromatin spots also occurs for rectangular patches. These results imply that a bipolar spindle has a limited segregation capacity that is determined by its ability to minimize the space occupied by its chromosomes (which, unlike beads affixed to a rigid surface, are free to move around). This implication explains the observation in frog egg extracts that pairs of juxtaposed spindles fuse into a single bipolar spindle, but fusion of more than two spindles often results in disorganized multipolar structures (Gatlin et al., 2009; Sawin and Mitchison, 1991). However, the fact that different cell types within the same organism have spindles of markedly different sizes suggests that the relationship between segregation capacity and spindle size must vary from one cell type to the next and that some cells are more efficient at packaging the same amount of chromatin into a smaller space.

In a demonstration of the broader utility of the micropattern approach, Dinarina et al. use the same spindle arrays to investigate intrinsic mechanisms of spindle assembly. Spindles assembled around small rectangular chromatin bead clusters are typically oriented with their interpolar axes lying perpendicular to the long axis of the chromatin. The authors find that these structures can be rotated away from their preferred axis by dynein-dependent traction forces between the poles of proximal spindles. These same pole-to-pole forces also promote the assembly of asymmetric half-spindles (Figure 1D). This demonstrates that interactions between adjacent spindles can perturb the normal mechanics that govern the assembly of individual spindles by providing a spatial configuration that allows polar traction forces to dominate. This finding may lend credence to force-balance models of spindle assembly.

The flexibility of the micropattern design system, coupled with the biochemical tractability of *Xenopus* egg extracts, should allow exploration of how specific proteins contribute to spindle assembly. Furthermore, this approach generates robust datasets that are well suited to the development of new models of spindle assembly and that provide a means to better test existing models. Needless to say, it will be interesting to see what bounty future harvests of spindle fields will yield.

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DNA Makes RNA Makes Innate Immunity

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Microbial DNA in the cytosol induces production of interferon-β (IFN-β) and an innate immune response. Chiu et al. (2009) now implicate cytosolic DNA-dependent RNA polymerase III as the DNA sensor linking DNA release by pathogenic bacteria and viruses in the host cell cytosol to IFN-β production and innate immunity.

DNA can be dangerous, particularly if it is in the wrong place. Accumulation of foreign DNA or self-DNA in the cytosol triggers an inflammatory response with the release of cytokines, such as interleukin-1β (IL-1β) and interferon-β (IFN-β). In the case of foreign DNA, this inflammatory response is important for innate immunity and host defense against bacterial and viral pathogens. An inflammatory response associated with self-DNA has been implicated in autoimmune diseases, such as systemic lupus erythematosus (SLE). Interestingly, this role for cytosolic DNA in inflammation was identified well before DNA's role in transcription was appreciated. In his Nobel Prize acceptance speech in 1908,

Mechnikov, the founding father of innate immunity, stated that nucleic acids could recruit protective phagocytes. And yet, despite the intervening 100 years, we still do not have a clear picture of how DNA triggers the innate immune response. In this issue of *Cell*, Chiu et al. (2009) provide new mechanistic insights into the signaling pathway that links the accumulation of cytosolic DNA to IFN-β production. They highlight a key role for DNA-dependent RNA polymerase III (Pol III). This ubiquitous enzyme normally is found in the nucleus, where it is involved in tRNA processing, but is also found in the cytosol, where its role has so far been unclear. A similar finding also has been reported by Ablasser et al. (2009) in *Nature Immunology*. Together, these exciting results identify a mechanism to sense microbial DNA, with the host cell Pol III acting as the sensor that transcribes the signal for recognition by the RNA sensor retinoic acid-induced gene I (RIG-I), leading to induction of IFN-β (Figure 1).

The new work began with a mystery: certain human cell lines transfected with double-stranded DNA (dsDNA) induced IFN-β production through activation of RIG-I, which senses dsRNA and single-stranded RNA containing a 5′ triphosphate (Hornung et al., 2006; Cheng et al., 2007; Schlee et al., 2009). So, how does RIG-I, an RNA sensor, recognize DNA in the cytosol? By comparing various types of DNA in cultured HEK293 cells, Chiu and colleagues were able to demonstrate that a cytosolic B form of dsDNA, poly(dA-dT)•poly(dA-dT), is able to activate RIG-I and to induce IFN-β production. Silencing of RIG-I or its signaling adaptor, the mitochondrial antiviral signaling (MAVS) protein, with small interfering RNAs (siRNAs) blocked this effect in cultured cells. Sequences containing GCAT or GC had no effect, nor did duplexes of polyA or polyT, leading the authors to conclude that the optimal form of cytosolic DNA for RIG-I activation and IFN-β production was AT-rich and 30 to 50 nucleotides in length.

The investigators then established a cell-free system so that they could identify protein players involved in this new pathway. First, they showed that HeLa cells respond to poly(dA-dT) DNA by production of IFN-β in a RIG-I-dependent manner. Cytosolic extracts of HeLa cells were prepared and fractionated with ammonium sulfate precipitation, dialysis, and, in a biochemical tour de force, eight steps of conventional chromatography. This purification enabled the authors to identify the missing link as Pol III. They confirmed this finding in three different ways: immunodepletion of Pol III, siRNA depletion of the POLR3F subunit of Pol III, and pharmacological inhibition of Pol III by the small molecule ML-60218. A key control experiment was the use of Sendai virus, which induces IFN-β production through viral RNA-dependent RNA polymerase and not Pol III.

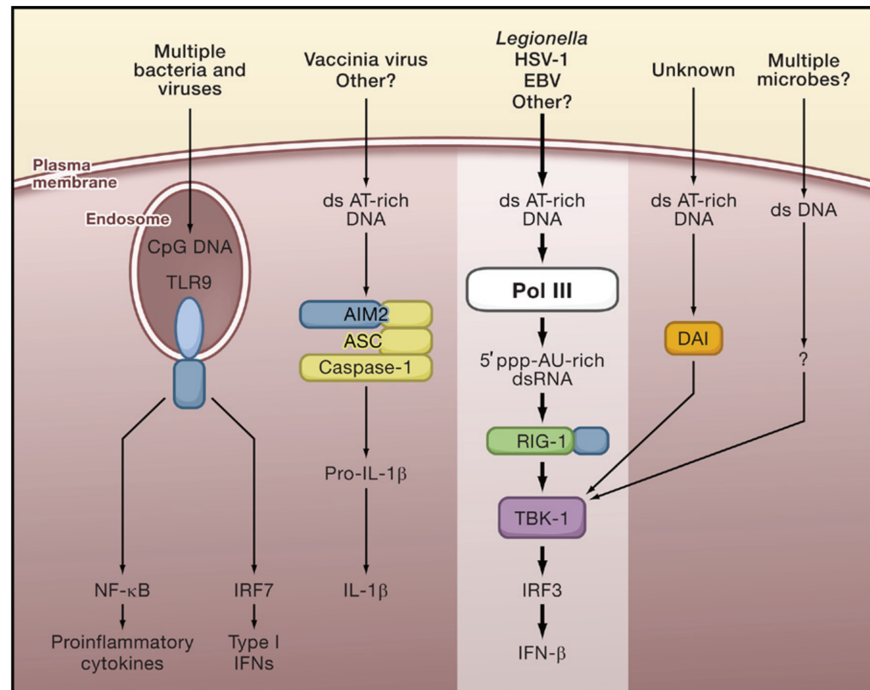

Figure 1. DNA Sensing and Innate Immunity

Shown are signaling pathways that detect microbial DNA in the host cell cytosol resulting in an innate immune response. In a newly discovered pathway (Chiu et al., 2009), a cytosolic DNA-dependent RNA polymerase III (Pol III) detects AT-rich double-stranded DNA (dsDNA) released by pathogens. Pol III converts this pathogen dsDNA into dsRNA that has a 5′ triphosphate. This RNA species is then sensed by the RNA sensor RIG-I, leading to IFN-β production and activation of innate immunity. Other well-established pathways sense microbial DNA in different cellular compartments, triggering production of different sets of cytokines. For example, Toll-like receptor 9 (TLR9) in endosomes senses CpG motifs in microbial DNA and activates signaling pathways, culminating in activation of NF-κB and IRF7 with subsequent production of proinflammatory cytokines and type I IFNs. Cytosolic DNA derived from vaccinia virus can be sensed by AIM2 in a complex with Asc and caspase-1, leading to the processing of pro-IL-1β to IL-1β. In certain cultured cell lines, AT-rich dsDNA can also be sensed by the protein DAI, which drives IFN-β production through activation of the protein kinase TBK-1. Finally, an as yet unknown receptor detects several types of microbial DNA, including plasmid-derived and GC-rich DNA in some cell types, leading to IFN-β production via activation of TBK-1.

Finally, the authors tested several pathogens—the bacterium *Legionella pneumophila* (the cause of Legionnaire's disease), herpes simplex virus type 1, and Epstein Barr virus (EBV)—that are known to activate RIG-I and MAVS. The Pol III inhibitor, ML-60218, blocked IFN-β production in response to all three pathogens, implicating this pathway in the innate immune response to key pathogenic bacteria and viruses. In the case of EBV, the authors went further, identifying two small noncoding dsRNA molecules containing 5′ triphosphate (EBER-1 and EBER-2) as products of host Pol III action and activators of RIG-1 and IFN-β production.

It has been known since 1977 that Pol III resides in the cytosol as well as the nucleus, yet it seemed to have no function in the cytosol (Jaehning and Roeder, 1977). It now seems that Pol III is in the cytosol to detect the DNA released by pathogenic bacteria and viruses and to

trigger innate immunity. The precise biochemical composition of cytosolic Pol III that enables it to specifically recognize AT-rich DNA is not yet known, but the 13 subunits in the complex are sufficient to provide this selectivity. It will be interesting to examine other bacteria, DNA viruses, and parasites such as *Plasmodium falciparum* (which causes malaria) to determine whether the Pol III/RIG-I pathway is a general mechanism for detecting pathogens and for triggering innate immunity.

The new findings also may be relevant to autoimmune diseases such as SLE. The noncoding RNAs of EBV, EBER1 and EBER2, produced by cytosolic Pol III, are normally sequestered in ribonucleoprotein particles (RNPs) in the nucleus. This is likely to be the mechanism by which EBV evades detection and destruction by the innate immune response. However, patients with SLE develop antibodies to these RNPs (Lerner et al., 1981; Rosa et al., 1981) suggesting that under certain circumstances these viral RNAs gain access to the cytosol triggering IFN-β production via RIG-I and resulting in an antibody response. Unfortunately, the antibodies recognize both microbial and self-antigens, thus contributing to SLE pathogenesis. Thus, Pol III and RIG-I may be potential new therapeutic targets for treating not only infectious diseases, but also SLE and other autoimmune disorders.

But this is not quite the end of the story. In some cell types, such as murine embryonic fibroblasts and primary bone marrow-derived macrophages, different forms of DNA including poly(dA-dT) seem to be able to induce IFN-β production in the absence of MAVS (Cheng et al., 2007; Ishii et al. 2006). From the Chiu et al. study, it appears that transformed cultured cell lines, such as HEK293 and HeLa, lack this MAVS-independent pathway. As the authors point out, this may well be the reason why such cell lines have proved useful in transfection studies, as they do not produce IFN-β in response to the introduction of DNA plasmids. Future studies will delineate the precise roles of the Pol III-dependent and Pol III-independent pathways for the induction of IFN-β and innate immunity in different cell types and during infection in vivo.

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Time's up: Bursting out of Transcription

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Many inducible genes are transcribed in bursts. In this issue, Degenhardt et al. (2009) report computational models that predict and validate patterns of stochastic gene expression.

The dynamic process of animal development and an organism's responses to a constantly changing environment are controlled with remarkable accuracy by transcriptional regulatory mechanisms. These regulatory mechanism are stochastic in nature, which leads to cell-to-cell variation in mRNA and protein levels (Raj and van Oudenaarden, 2008). This apparent paradox between stochasticity and determinism exists in all organisms from bacteria to humans. An integral component to this randomness is transcriptional cycling (transcriptional bursts) in which a gene is switched between the active and the inactive states. However we do not know what determines the length and intensity of each cycle of transcription, although both length and intensity remain constant during development (Chubb et al., 2006). Furthermore, it is unknown how these stochastic bursts are synchronized across a population of cells to produce cycling. In this issue of *Cell*, Degenhardt et al. (2009) introduce a mathematic model explaining how individual cycling cells are synchronized in a population of cells to produce oscillating patterns of mRNA accumulation.