how the transport of warm ocean currents continued to evolve in the subsequent tens of millions of years, and how such changes relate to the falls in global temperatures over the past 20 million years.

Taken together, these papers[2–4] resolve some enigmas about the climatic evolution of the Arctic. But we still need to sort out why the intensity of glaciation stepped up around 14 million years ago and increased further around 3 million years ago, because neither step was tied to decreases in atmospheric CO_2. Also, given that the modelled effect of CO_2 is not sufficient to explain the Palaeocene super-greenhouse and some other (possibly greenhouse) agent is required, then the demise of this other factor may also have affected the growth of ice sheets and the global cooling.

In all this, there's a particular challenge for those involved in climate modelling. If they can incorporate the processes causing the hot poles in the past, we will have even greater confidence in their predictions for the future. ■

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VIROLOGY

HIV goes nuclear

Min Li and Robert Craigie

HIV-1 replicates itself by integrating into its host cell's DNA. Studies in cell culture reveal that nuclear-membrane proteins aid engagement of the viral DNA with that of its host before integration.

Rather than going to the trouble of replicating its own genome, human immunodeficiency virus type 1 (HIV-1) inserts a DNA copy of its genome into that of its host so that the virus is reproduced during host-cell division (Fig. 1). The virus genome consists of RNA, so a DNA copy must first be made from the RNA template. The newly synthesized viral DNA forms part of a large complex in the cytoplasm of an infected cell, the pre-integration complex (PIC). This contains the viral integrase enzyme that splices the viral DNA into cellular DNA, as well as other viral and cellular proteins. To reach the cellular DNA, the PIC must cross the nuclear envelope that separates the cytoplasm and nucleoplasm. On page 641 of this issue, Jacque and Stevenson[1] show that HIV-1 PICs target a specific nuclear-membrane protein called emerin, and that this is required for the viral DNA to engage with the host DNA before integration.

Unlike many viruses, HIV-1 and other lentiviruses can infect cells that are not dividing, so the viral DNA must cross the intact nuclear envelope to gain access to the cellular chromatin (the DNA and associated proteins that package it into chromosomes). This presents a formidable challenge, because the PIC is huge on a molecular scale — far larger than the diameter of nuclear pores. Thus, nuclear entry may be facilitated by first jettisoning some components of the PIC. Many protein components of the PIC carry signalling sequences that enable them to enter the nucleus, but the roles of these proteins and the mechanism by which the PIC is imported into the nucleus remain controversial. Many cellular proteins have been reported to associate with PICs[2], but in most cases the functional consequences are unclear.

The nuclear envelope is composed of the inner and outer nuclear membranes, embedded pore complexes, and the lamina and associated proteins[3]. The nuclear lamina, which lies just inside the inner nuclear membrane, is a network of lamin proteins that, as part of the nuclear 'matrix', provides structural support for the nucleus. Proteins associated with the lamina include the lamin B receptor (LBR), lamin-associated polypeptides 1 and 2 (LAP1 and LAP2), emerin and MAN1. LAP2, emerin and MAN1 share a conserved structural domain (called the LEM domain) that binds to a protein known as the 'barrier-to-auto-integration' factor (BAF). BAF was first identified as a factor that blocks the self- destructive integration of murine leukaemia virus (MLV) DNA into its own genome *in vitro*[4]. This factor is a non-specific DNA-binding protein that can bridge strands of duplex DNA and may link chromatin to LEM-domain proteins at the nuclear envelope[5]. BAF is required for progression through the cell-division cycle[6], and lack of BAF causes dividing cells to die[7].

Jacque and Stevenson[1] examined the ability of HIV-1 to infect cells in which individual nuclear-envelope-associated proteins had been selectively depleted using a method called RNA interference (RNAi). This technique selectively targets messenger RNAs for degradation, stopping production of the encoded protein. The authors shrewdly studied the roles of the nuclear-envelope proteins in primary macrophages, one of the immune cell types infected by HIV. These cells do not divide, so this strategy bypassed the lethal effects of loss of BAF, and sidestepped potential cell-division roles of the other nuclear-envelope proteins tested.

Depletion of either emerin or BAF severely impaired HIV-1 replication in infected macrophages. Notably, expression of these proteins using RNAi-resistant mRNAs restored replication, supporting the conclusion that the defect was the direct consequence of depleting each protein. The emerin-depleted cells were fully susceptible to infection with a different

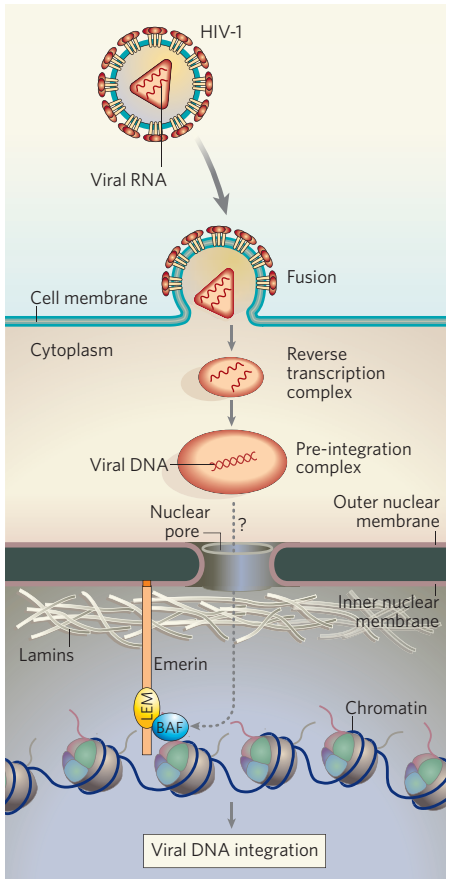

Figure 1 | HIV-1 integration into the host genome. As HIV-1 is an RNA virus, to integrate into the genome of its host cell it must first be converted into DNA by reverse transcription. The viral DNA then forms part of the pre-integration complex with several proteins, including the integrase enzyme that splices it into the host DNA. Jacque and Stevenson[1] show that once the pre-integration complex has entered the nucleus (by an unknown mechanism), it targets a specific nuclear-membrane protein called emerin. Emerin forms part of the lamina beneath the nuclear membrane, where lamin proteins provide structural support for the nucleus. The host DNA may be associated with the lamina through the BAF protein, which binds to LEM-domain proteins such as emerin. Jacque and Stevenson report that both emerin and BAF are required for the viral DNA to integrate into the host DNA.

retrovirus (MLV), further indicating that the replication defect is specific to HIV-1 and not due to a widespread disruption of the normal cell physiology. Replication of MLV required BAF and LAP2α, but not emerin[1,8]. So it would seem that, although both retroviruses recruit BAF, each virus also enlists different LEM-domain proteins.

What is the viral replication defect in cells depleted of either emerin or BAF? In these cells, viral DNA was synthesized at normal levels but failed to integrate into the cellular genome. Biochemical subcellular fractionation experiments indicated that depletion of emerin or BAF did not prevent the viral DNA from entering the nucleus, but that the viral DNA became associated with different nuclear fractions from normal. In control macrophages, most of the viral DNA was associated with the soluble chromatin fraction, whereas in cells depleted of BAF or emerin it was mainly in the insoluble nuclear-matrix fraction. The identical infectivity defects caused by depletion of either emerin or BAF suggest that these proteins have a cooperative role in HIV-1 infection, consistent with the known interaction between BAF and the LEM domain of emerin.

The mechanism by which emerin and BAF facilitate the proper nuclear localization of the HIV-1 PICs remains unknown. The association of emerin with the PIC depends on BAF, which probably interacts with the viral DNA. But how does emerin then influence the association of the HIV-1 PIC with the host chromatin? It may be that the work required to answer this question will also uncover other features of nuclear architecture. Although the organization of chromatin has been extensively studied at the level of the nucleosome (the smallest unit of DNA packaging), the global organization of chromatin within the nucleus is not well understood. However, the nucleus is clearly both highly compartmentalized and dynamic, and chromatin is intimately associated with the nuclear envelope. Perhaps we should not be surprised that there is more to accessing chromatin than simply crossing the nuclear envelope. ■

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DOWN'S SYNDROME

Critical genes in a critical region

Charles J. Epstein

The NFAT transcription factors activate the expression of many genes involved in the immune response and the development of a variety of tissues. They have now been implicated in Down's syndrome.

Down's syndrome is most commonly caused by the presence of an extra copy of the major portion of human chromosome 21. But how does the presence of an extra set of the roughly 200–300 genes on the chromosome give rise to the many abnormalities that characterize the condition? Because the pattern of abnormalities is so specific, one theory is that the 1.5-fold increase in the expression of some, if not all, of these genes is responsible[1].

In this issue, papers by Arron *et al.* (page 595)[2] and Gwack *et al.* (page 646)[3] implicate two genes in the so-called Down's syndrome critical region (DSCR), a small segment of human chromosome 21, in causing the abnormalities found in Down's syndrome. Using diametrically opposed approaches, the groups reached this conclusion by a process that began with an interest in a family of four gene-regulatory factors called NFATc (for 'nuclear factor of activated T cells').

The regulation of various developmental pathways and of the immune response relies on processes that are activated by the entry of calcium into the cell, and the NFAT signalling pathway mediates many of these processes. Following the influx of calcium, phosphate groups are removed from NFATc factors in the cytoplasm by the enzyme calcineurin. This allows NFATc to enter the nucleus and activate its target genes. However, once in the nucleus, NFATc can have phosphate groups added back to it by a kinase enzyme (phosphorylation), forcing it to return to the cytoplasm and halting its effects on the genes (Fig. 1a).

Arron *et al.*[2] came upon the possibility of a connection between the NFAT system and Down's syndrome by the serendipitous observation that mice lacking NFATc2 and NFATc4 have abnormalities of the skull and jawbone. These deformities are similar to those observed in Down's syndrome and in two mouse models of Down's syndrome (called Ts65Dn and Ts1Cje) that have an extra copy of part of the mouse chromosome most similar to human chromosome 21 (that is, they are trisomic)[4]. In addition, these and other mice lacking various NFATc family members, either singly or in combination, display abnormalities that are highly reminiscent of Down's

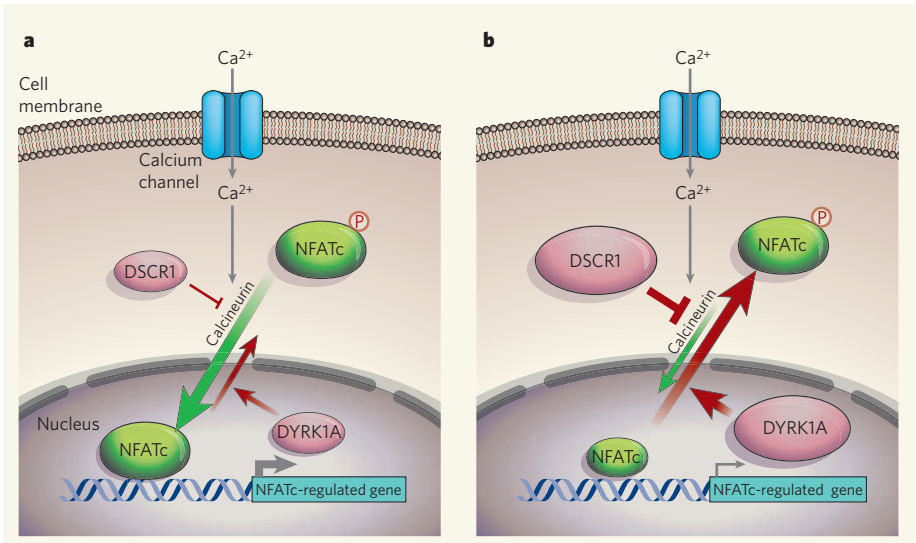

Figure 1 | NFAT signalling and Down's syndrome. Calcium signalling through the NFATc pathway mediates many developmental processes and the immune response. **a**, The entry of calcium ions into the cell activates the enzyme calcineurin to remove phosphate groups (P) from NFATc factors in the cytoplasm, allowing NFATc to enter the nucleus and activate its target genes. However, once in the nucleus, the NFATc can be phosphorylated, and so returns to the cytoplasm. Arron *et al.*[2] and Gwack *et al.*[3] implicate the DSCR1 and DYRK1A proteins in regulating the levels of NFATc phosphorylation. **b**, The genes encoding DSCR1 and DYRK1A are found in the 'Down's syndrome critical region' of human chromosome 21, which has an extra copy in people with Down's syndrome. The increased expression of *DSCR1* and *DYRK1A* disturbs the balance of NFATc phosphorylation, so that most of the protein is found in the cytoplasm[2,3]. Thus, NFATc-dependent genes will not be properly regulated, which could markedly affect development. (Modified from Arron *et al.*[2], Supplementary Figure 1.)