measurements that depend on diffusive motion — measurements of particle sizes and diffusion coefficients, for example, and of the behaviour of critical fluids. Indeed, any fluid measurements performed in space must be interpreted very carefully, to fully take into account the effects of the fluctuations.

Moreover, some manifestations of diffusive motion that are well established on Earth may be profoundly changed in microgravity. Even the mixing of fluids may be appreciably affected, by a change in the timescale of homogeneous mixing. Further surprises are probably to be found in diffusion-driven phenomena without gravity. □

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Virology

Illicit viral DNA

Robin A. Weiss and Paul Kellam

More than 20 years ago, the late Victor Zhdanov at the Ivanosky Institute of Virology in Moscow published a remarkable paper[1] claiming that complementary DNA copies of RNA viruses such as measles and polio occurred in retrovirus-infected cells. The observations raised eyebrows at the time, because promiscuous cDNA synthesis seemed to run counter to everything known about viral replication. But the data were neither confirmed nor refuted, and were soon forgotten.

On page 298 of this issue[2], Rolf Zinkernagel's group resurrects the issue posed by Zhdanov's results. Klenerman *et al.*[2] show that cDNA fragments of the RNA-replicating lymphocytic choriomeningitis virus (LCMV) form in mice and in murine and hamster cells in culture which express retroviral reverse transcriptase, the enzyme which uses an RNA template to synthesize DNA. The formation of LCMV cDNA is inhibited by azidothymidine (AZT), confirming that reverse transcriptase activity is involved. There is, of course, ample evidence for reverse transcription having occurred in evolution, with the formation of processed DNA or pseudogenes from RNA[3]. But the LCMV cDNA is a case of a non-retroviral RNA being caught red-handed in the act of seemingly illicit DNA synthesis.

Many viruses of animals, plants and bacteria carry their genetic information in the form of RNA. With the exception of retroviruses, they replicate through RNA intermediates, so that no viral DNA sequences are synthesized at any stage of the viral life cycle. LCMV is such a virus[4] (and belongs to the arenavirus family, which also includes the dreaded Lassa fever virus). The RNA in LCMV virus particles is composed of two 'ambisense' single-stranded molecules, large and small. Part of each molecule has the same coding sense as messenger RNA from which the viral proteins are translated, and part is complementary to mRNA. LCMV encodes and packages an RNA polymerase which makes complementary RNA from the genomic RNA template. The viral polymerase is not known to have reverse transcriptase activity. That is why it is so surprising that Klenerman *et al.* detect DNA sequences homologous to viral RNA.

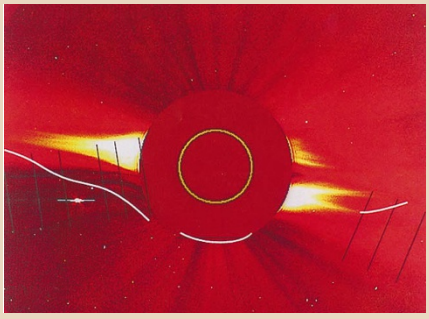
When outlandish claims are based on detection through the polymerase chain reaction (PCR), the sceptic's initial reaction is to suspect false-positive data. After all, a laboratory in which cloned viral sequences are handled, or where reverse-transcriptase PCR is routinely used to detect viral RNA, is just the right environment for error, as we all know to our cost. But Klenerman *et al.* appear to have performed all the appropriate controls to guard against contamination.

One reason why they investigated the presence of cDNA was to try to solve an immunological puzzle — mice which have cleared all evidence of previous LCMV infection continue to show strong immune responses as if some viral antigen persisted in the animals. The authors therefore speculate that the cDNA might act as a naturally produced form of DNA vaccine that produces antigen. This is difficult to conceive for cDNA lacking promoter sequences for expression. However, if any of the cDNA sequences were to integrate downstream from cellular gene promoters or within an open reading frame, a low level of specific peptides might be expressed that would be sufficient to load major histocompatibility antigens in antigen-presenting cells, and thereby elicit an immune response.

Klenerman *et al.*[2] detected LCMV cDNA in mouse and hamster cells expressing reverse transcriptase activity. But it was not found in a variety of other cells without such activity, or in reverse-transcriptase-positive guinea-pig cells. Further work is required to find out whether retroviral reverse transcriptase is responsible for the LCMV cDNA synthesis, or whether some other cellular component in mouse and hamster cells confers a reverse transcriptase activity upon the LCMV RNA polymerase. One of the reverse-transcriptase-negative cell lines should be infected with amphotropic murine leukaemia virus and then superinfected with LCMV to see whether cDNA

Solar physics

Galileo through the Sun's streamers



The Galileo satellite, on its tour of Jupiter's moons, has provided a remarkable bonus for solar physicists. In January, the Sun obstructed our line of sight to the distant probe. By monitoring radio signals from Galileo as they passed through the Sun's corona, astronomers have solved an old problem about the origin of the solar wind. It has long been known that the wind has two distinct components, slow and fast. But where on the surface of the Sun do they originate?

This image (Habbal, S. R. *et al. Astrophys. J.* 489, L103–L106; 1997) is a white-light view obtained using the Solar and Heliospheric Observatory (SOHO). The solar disk is blanked out, revealing the hot, inner coronal regions and filamentary structures known as streamers. Jupiter is the bright point to the left of the solar disk.

Galileo's apparent passage behind the Sun is marked by the straight black lines, which show where the slit of a spectrometer on SOHO was positioned to make ultraviolet measurements of the corona, simultaneous with the radio transmissions. The UV spectra give a rough indication of the Solar wind speed, and the 94 km s^{-1} contour is shown in white. Scintillation of Galileo's radio signal also indicates wind speed, importantly with very high spatial resolution: on one occasion, the scintillation increased markedly (a sign of the slow wind), and, by no coincidence, a streamer stalk intercepted the line of sight to the probe at the same time. It seems that the slow wind comes from streamers, and the fast wind comes from the whole surface — not, as had been thought, only from the polar regions.

Eventually, these results may help solve the biggest mystery of the solar corona — the nature of its heating mechanism.

Karen Southwell

only appears in the presence of the enzyme.

If retroviral reverse transcriptase really is the culprit, how does the enzyme gain access to its illicit RNA template, and what primer is used to initiate reverse transcription? In retroviruses, various transfer RNA molecules act as primers by specifically annealing to primer binding sites in the viral genome. LCMV sequences could be analysed for potential primer binding sites or for sequences that may allow LCMV RNA to be packaged in retrovirus particles. This might explain why LCMV cDNA synthesis occurs only in some cells which are positive for reverse transcriptase, as the enzyme is not present in the cytosol in active form, and cross-packaging may be specific to certain types of retrovirus. Alternatively, reverse transcriptase might be incorporated into LCMV particles, which contain ribosomal 28S and 18S RNA, as well as low-molecular-weight 4–7S cellular components[4] which may include tRNA.

A host of questions remains. How general might the phenomenon described by Klenerman *et al.*[2] be? Is it solely limited to retroviral reverse transcriptases, or does it encompass related polymerases of, say, the hepatitis B virus family? Is LCMV a special case because of cross-packaging of RNA or of reverse transcriptase, or is it a convenient marker RNA sequence as no homologous DNA exists in uninfected cells? Was Zhdanov right after all? Will humans persistently infected with the retrovirus, human immunodeficiency virus, make cDNA forms of other, non-retroviral RNA viruses? Could cDNA synthesis help to explain why it is so technically difficult to detect negative-strand genomes of hepatitis C virus[5] (as evidence for *de novo* replication) — if the 'false' positive reverse transcriptase PCR results that are so common was natural cDNA? How long are the cDNA transcripts of LCMV, and do they integrate into the host genome?

Clearly, there is much to keep molecular virologists occupied arising from these new observations. They present a phenomenon that might have consequences in understanding the reshuffling of genetic material, and the pathogenesis of double virus infections. □

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Evolutionary biology

More mutations in males

Kate Lessells

Mutation is the ultimate source of all genetic novelty and is therefore a key process in evolution. As early as 1947, Haldane[1] suggested that, if copying mistakes during cell replication were a major source of mutations, mutation rates should be higher in males than in females because of the greater number of cell divisions involved in spermatogenesis compared with oogenesis. Writing in *Nature Genetics*, Ellegren and Fridolfsson[2] confirm Haldane's prediction by analysing the molecular evolution of a pair of genes on the avian sex chromosomes.

Chromosomal sex determination is similar in birds and mammals except that in birds it is the males that have two identical sex chromosomes (ZZ) and the females that are heterogametic (ZW). W chromosomes are passed from one generation to the next only in eggs, whereas Z chromosomes are transmitted one-third of the time in eggs and two-thirds of the time in sperm. So if genes mutate more frequently in males, Z-linked genes should have a higher mutation rate than W-linked genes, and their relative mutation rates will bear a simple mathematical relationship to the relative mutation rates in males and females[3]. Genes on sex chromosomes can therefore be used to estimate relative mutation rates in males and females.

Ellegren and Fridolfsson investigated nucleotide-sequence evolution in the *CHD* genes, different versions of which are found on the W (*CHD-W*; refs 4–6) and Z (*CHD-Z*; ref. 7) chromosomes. These genes are the only homologous pair of coding genes that have been identified on the avian sex chromosomes so far. The analysis of homologous pairs of coding genes has two advantages. First, it avoids the possibility that exists for non-homologous sequences of mutation rates differing for reasons other than the sex of the parent[8]. Second, it allows nucleotide sequences to be unambiguously aligned and sequence differences to be identified[3].

By comparing nucleotide sequences from several bird species, the authors estimated the rate of evolutionary change in each of the *CHD* genes. However, nucleotide pairs that affect the proteins encoded by the genes will be subject to selection, and this will confound any attempt to estimate mutation rate from differences in sequences[3]. Ellegren and Fridolfsson got around this problem by analysing both synonymous nucleotide differences (where the triplet containing the mutation still encodes the same amino acid, so that the protein produced is unchanged) and the sequence of an intron (a non-coding length of DNA within the coding gene). The rate of sequence change was 2.9 (synonymous mutations) or 4.6 (intron) times higher for *CHD-Z* than for *CHD-W*, implying that the mutation rate is 3.9 or 6.5 times higher in males than in females.

This is not the first study to suggest that mutation rates are higher in males. A similar logic has been applied to the sequences of genes on the X and Y chromosomes of primates[8,9] and rodents[9]. But the higher rate of change in Y-linked compared with X-linked genes does not unequivocally demonstrate a higher rate of mutation in males because there is an alternative explanation[10]: mutation rates reflect in part a trade-off between the benefits of a low mutation rate and the costs of the molecular machinery needed to ensure accurate replication. Mutations in genes on the paired sex chromosomes (X in mammals, Z in birds) are more disadvantageous than mutations in autosomal genes because deleterious recessive mutations will be exposed when hemizygous (that is, occurring on the X or Z chromosome opposite a non-coding Y or W chromosome) but not when heterozygous. So selection should favour more accurate replication, and hence a lower mutation rate, of the X or Z chromosome. In mammals, the two explanations — more mutations in the sex with more divisions in gametogenesis (the male), and fewer mutations on the paired sex chromosome — cannot be distinguished because they both predict higher rates of change in Y-linked than in X-linked genes. In birds, males are homogametic, so the higher mutation rate of *CHD-Z* compared with *CHD-W* can only be explained by a greater number of cell divisions in males.

If mutations do occur primarily during cell division, the relative mutation rates in males and in females should equal the relative numbers of cell divisions during spermatogenesis and oogenesis. This is not known in birds, but has been estimated in humans: there are about 33 divisions from zygote formation to oogenesis, and 205 from zygote formation to spermatogenesis (in a 20-year-old man)[9]. The ratio of these two numbers (6.2) is close to the ratio of the mutation rates in male and in female higher primates (6; ref. 9).

A higher mutation rate in males than in females implies that point mutations are largely associated with cell division and are not caused by other factors such as methylation or oxygen radicals that are unrelated to replication. This implication touches on diverse areas of evolutionary biology. First, it provides an explanation for the effect of generation time on the rate of molecular evolution[3,8]. Second, it suggests that devel-