so patient commitment to safe sex practices will be an important adjunct to STI. It is not clear whether superinfection is only a risk during treatment interruption: more studies on this are needed.

Altfeld *et al.*'s work[1] is a beautiful illustration of the power of modern techniques to explore the minute details of a virus-specific immune response. But the effort required will preclude studies of large numbers of patients. Certainly, this single-patient analysis raises many questions, but whether the news is bad, neutral or even good remains to be seen. Although causing a brief pause for thought, nothing here should slow or divert efforts to develop an HIV vaccine. ■

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Circadian rhythms

The cancer connection

Michael Rosbash and Joseph S. Takahashi

The *Per2* gene is a core component of the circadian clock in mammals. It now seems that the mouse *Per2* gene is also involved in suppressing tumours, through other genes that affect cell proliferation and death.

If the mantra in real estate is 'location, location, location', in genetics it would be 'phenotype, phenotype, phenotype'. There is simply no substitute for a detailed phenotypic analysis of a mutant strain (study of the overt manifestation of a mutated gene in the organism). This has the potential to reveal unanticipated — and sometimes truly surprising — relationships between genotype and phenotype, or between a primary phenotype and a secondary one. Such was the case for an analysis published recently in *Cell* by Lee and colleagues[1].

The organisms under study here were mice in which both copies of the *mPer2* gene were mutated — a genotype shown previously[2] to cause a strong defect in circadian rhythms. Most organisms have endogenous 'clocks' that control rhythms of physiology and behaviour with roughly 24-hour (circadian) periodicity. But the period is shortened, and rhythmicity is lost, in the *mPer2* mutant mice. This is reminiscent of the effects of the original *perS* or *per0* mutations in fruitflies, described in the 1971 landmark paper from Konopka and Benzer[3].

By observing their mutant mice for a couple of years, however, Lee and colleagues[1] made an unexpected discovery: the animals were unusually cancer prone. At six months of age they began to show excessive cell proliferation in the salivary glands, as well as teratomas — tumours that originate from germ cells and comprise a mix of cell types. Thirty per cent of the mutant mice died before the age of 16 months, half of these from spontaneous lymphomas. In contrast, such lymphomas were first found in normal mice at the age of 20 months, a highly significant difference. The mutant animals were also more sensitive to γ-radiation, as indicated by premature hair greying and hair loss, and an increased rate of tumour formation — this last effect stemming, at least in part, from a decreased likelihood of cell death (apoptosis) in response to radiation.

So, what could be the story here? The current picture of the central circadian clock in animals is of a self-sustaining transcription–translation feedback loop, involving the transcription of key clock genes, their translation into protein, and the proteins' repression of transcription of the same key genes[4] (as well as of downstream, clock-controlled genes). In fact, given the importance of post-translational mechanisms — such as protein phosphorylation and turnover — and the lack of translational control in the current picture, it might be more accurate to describe it as a macromolecular feedback loop. In any case, the mPer2 protein is a key clock component: it contributes to the circadian regulation of transcription of both *mPer2* and downstream genes[5].

All of which begs the question: is there a tight relationship between γ-radiation and clock genes? And could disruption of circadian transcriptional regulation cause the defects in cell proliferation and death (together termed cell growth) seen even in the absence of γ-radiation in *mPer2* mutant mice? In other words, is there a transcriptional cascade from clock genes, to downstream growth-control genes, to growth-effector genes? The answers all appear to be yes.

Lee and colleagues' results show that, in normal mice, the expression of several core clock genes was rapidly and potently upregulated in the liver in response to γ-radiation. But in the mutants this response was absent or severely attenuated. Even more surprising was the authors' analysis of a few key genes concerned with cell growth. They found that expression of the *Myc* gene, as judged by levels of its messenger RNA, was circadian in wild-type liver; but in the mutants the expression pattern was modestly shifted and levels of *Myc* mRNA were dramatically increased. Moreover, experiments in cultured cells suggested that *Myc* transcription is directly regulated by the circadian clock. The authors also looked at the expression of *cyclin D1* and *Gadd45α*, two *Myc*-regulated mRNAs, and found that the levels of both fluctuated in a circadian pattern in wild-type livers; in the mutants, both patterns were altered.

So Lee and colleagues propose that the key effect of inactivating *mPer2* is to derepress *Myc* expression, leading to excessive cell growth and tumour formation. The effect is exacerbated by γ-radiation, which normally upregulates clock genes and thereby presumably leads to *Myc* repression. This fails in the *mPer2* mutants. If these proposals are true, there are some testable predictions. First, overexpresssing *Myc* in an otherwise normal genetic background should have the same growth-promoting effects as mutating *mPer2*. Second, and more important, inhibiting *Myc* expression should suppress tumour formation in *mPer2* mutants.

One caveat is that all of these experiments were performed under conditions of 12 hours' light, 12 hours' darkness, so it could be that the mRNA cycles were merely light-driven, not clock-driven. In this context, it is notable that *Myc* and *Gadd45α* were not identified as cycling genes (although *cyclin D1* was) in three out of four microarray studies of liver mRNAs[6–9]. But the marked effects of the *mPer2* mutation suggest that, at the very least, there is a strong connection between cell growth and the circadian clock. The discrepancy also reinforces the importance of taking microarray data — especially negative data — with a pinch of salt. In our opinion, careful biochemical analyses are more credible.

More generally, the new results[1] have brought into proximity two previously disparate fields of study: circadian rhythms and cell-growth control. One reason why they have hitherto been infrequent bedfellows is that the mammalian circadian rhythm field has historically focused on the brain and, more narrowly, on the suprachiasmatic nucleus — the region of the hypothalmus that is essential for directing cycles of loco-

motor activity. Most adult neurons do not divide yet manifest potent molecular rhythms, suggesting that these rhythms are controlled separately from cell division. A similar conclusion stems from work on rat fibroblast cells, where molecular rhythms persist even if cell division is blocked[10]. And classical work in microbes indicates that rapid cell division can be completely uncoupled from the 24-hour timing of the circadian system in the same cells[11].

On the other hand, cell division in microbes can be driven by the circadian cycle when the periodicity of division is not far from 24 hours. This suggests that the circadian system is important for proper growth control, and is consistent with the apparent circadian regulation of cell proliferation and apoptosis[1]. Moreover, the striking time-dependent response of wild-type mice to γ-radiation[1] reinforces the potential importance of circadian principles in cancer and cancer therapies. It seems that cancer can be a direct consequence of the absence of circadian regulation. Perhaps this applies to other diseases, too. For instance, shift workers tend to have increased health problems. These could result indirectly from disruption of physiological systems that are under circadian control — but there might also be a direct connection to the clock. ■

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Applied physics

Strong magnets by self-assembly

David J. Sellmyer

Newly developed nanomaterials are proving useful in many fields, but materials that make strong permanent magnets are difficult to devise. Progress has been made using a self-assembled mixture of nanoparticles.

Controlled structuring of materials at the nanoscale can enhance some of their properties and widen their range of applications. Magnetic materials, such as recording media, field sensors and memory devices, are advancing rapidly in terms of their miniaturization, sensitivity and other figures of merit. But progress in producing permanent magnets has been limited by the difficulty of finding new compounds with the necessary properties. On page 395 of this issue, Zeng *et al.*[1] show how self-assembly of mixtures of magnetic nanoparticles makes it possible to fabricate materials with excellent magnetic properties. The process of chemical synthesis and the resulting nanostructures, with magnetic coupling between nanoscale grains in the composite material, are impressive.

The figure of merit by which permanent-magnet materials are judged is the energy product — a measure of the maximum magnetostatic energy that would be stored in free space between the pole pieces of a magnet made from the material in question[2]. The energy product depends on the area of the 'hysteresis loop' (Fig. 1). A typical hysteresis loop arises from plotting the magnetization of the material as the applied magnetic field is varied — the response of the materials follows two distinct paths on magnetization and demagnetization. As well as the saturation point (maximum magnetization), the hysteresis loop is characterized by the 'coercivity' of the material, which is the reverse-field strength needed to reduce the flux density to zero. To obtain a large energy product requires large magnetization and large coercivity.

The largest room-temperature magnetization for a material — a $Fe_{65}Co_{35}$ alloy — corresponds to an internal flux density or magnetic induction of about 24 kilogauss. Theoretically, the upper bound on the energy product for the material (proportional to the saturation magnetization squared) is 144 MGOe, for induction measured in gauss (G) and field in oersted (Oe). But in practice, the Fe–Co alloy does not have such a large energy product because it is a soft magnet — that is, its coercivity is actually fairly low and a small reverse field is sufficient to reduce the magnetization to zero.

The largest energy product observed in nature, for the compound $Nd_2Fe_{14}B$, is 56.7 MGOe (ref. 3). $Nd_2Fe_{14}B$ has a complex structure containing 68 atoms in its unit cell. The Nd–Fe–B class of magnets owes its position as the highest-energy magnet to its high magnetization (mainly from Fe ions) and to its tetragonal structure and Nd ions, which together produce a high degree of magnetic anisotropy. This anisotropy in turn causes high coercivity.

Is there hope of raising the maximum energy product observed to values approaching the upper bound of 144 MGOe? Despite strenuous efforts, a compound with the necessary properties has eluded researchers. But, about a decade ago, an idea emerged that brought new hope. This is the concept of 'exchange coupling' between a hard (high-coercivity) material and a soft (low-coercivity) material with a large magnetization. In a two-phase mixture of such materials, exchange forces between the phases mean that the resulting magnetization and coercivity of the material will be some average of the properties of the two constituent phases[4]. But for the exchange coupling to be effective, the relative sizes of the grains of the two materials must be chosen carefully: Kneller and Hawig[5] showed that the characteristic dimensions of the soft phase cannot exceed about twice the wall thickness of magnetic domains in the hard phase. Typically, this limits the soft phase to grains of about 10-nm diameter. Similarly, the hard phase must have dimensions of this order or the volume fraction of the soft phase will be rather low, thus limiting the magnetization of the composite.

Skomski and Coey[4] showed that in an ideal exchange-coupled magnet, consisting of aligned grains of $Sm_2Fe_{17}N_3$ and $Fe_{65}Co_{35}$, the theoretical upper bound on the energy product is about 125 MGOe. But in the real world, attempts to fabricate two-phase nanostructures with a high energy product have had only limited success. Bulk magnets produced by subjecting the two-phase material to ultra-fast cooling and then annealing, or by mechanical milling, have not achieved energy products beyond about 20 MGOe (ref. 6). There are several difficulties to be overcome: controlling the material structure at the nanoscale, especially to create uniform grain sizes of about 10 nm; aligning the hard grains sufficiently; and ensuring effective exchange coupling between the two phases in all grains through a homogeneous distribution.

In fact, higher energy products have been obtained in thin-film materials[7] based on iron–platinum compounds. In these, perpendicular anisotropy (and hence high coercivity) is achieved by first preparing nanoscale Fe–Pt multilayers, and then applying a specific thermal processing technique. Unfortunately, however, this technique is not appropriate for making practical bulk magnets. All in all, the work so far has shown the nanostructuring challenges to be formidable.

But now Zeng *et al.*[1] have devised a method of chemical synthesis with the