colleagues' data, we must also consider whether fasting and changes in diet might be changing leptin levels, thus altering the function of T cells.

The implication of this new work is that leptin drives the activity of pro-inflammatory, self-reactive T cells, and that starvation, which reduces leptin production, changes the pattern of cytokines generated and the disease-inducing potential of the T cells. So, the authors show that instead of producing pro-inflammatory cytokines, T cells from the starved mice generate anti-inflammatory cytokines, which generally inhibit and regulate organ-specific autoimmune diseases. The idea that leptin could also have a significant role in multiple sclerosis is strengthened by studies of the genes expressed in the brains of patients with this disease: leptin and related genes are expressed more than usual[8].

In the context of the whole animal, however, there is still much to understand about the potential interactions between fat metabolism and immunity. The importance of these questions to multiple sclerosis is highlighted by another recent study[9] of EAE. This work found that drugs of the statin family, which inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase and reduce cholesterol levels, are also beneficial to animals with EAE, again by altering the cytokine profile of self-reactive T cells. Another enzyme — stearoyl coenzyme A desaturase-1, which is involved in fatty-acid synthesis — is thought to contribute to leptin's effects on metabolism[10].

Although these enzymes are on different biosynthetic pathways, their genes are regulated by a common set of gene-transcription factors (adipocyte-determination differentiation-dependent factors, also called sterol-regulatory-element-binding proteins)[11] that also regulate the leptin gene[12]. So the coordinated control of these different metabolic pathways and leptin could be tied, by transcription factors, both to each other and to aspects of immune function.

In a broader context, these results illustrate once again the trade-off between resistance to infection and susceptibility to autoimmunity. This view is supported by genetic evidence that the regions on chromosomes that influence susceptibility to infection overlap with those that influence susceptibility to autoimmune disease[13]. The demonstration[2] that starvation can stop EAE reminds us how profoundly our state of immunity is also contingent on our relationship with the environment. Adequate nutrition supports an immune response poised to repulse pathogens. It may also be the best substrate for the seed of autoimmunity to take root. ■

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Microfluidics

DNA amplification moves on

Andrew J. deMello

The polymerase chain reaction is widely used to amplify samples of DNA for genetic analysis, and fast, high throughput is the ideal. Microscale, chip-based devices are now proving themselves in this arena.

The advent of the polymerase chain reaction (PCR) has hugely accelerated the progress of studies on the genetic structure of a diversity of organisms. PCR is an enzyme-catalysed reaction that allows any nucleic acid sequence to be generated *in vitro*, and in abundance —hence the term 'DNA amplification'. First reported[1] in 1986, PCR has since become a requisite tool in basic molecular biology, genome sequencing, clinical research and evolutionary studies. In an attempt to improve the speed and efficiency of amplification, several groups[2–4] have created microfabricated systems for PCR, and, in *Analytical Chemistry*, Pierre Obeid and colleagues[5] report the fabrication of a microfluidic chip that performs both PCR and reverse transcription (the synthesis of DNA using RNA as a template).

The reason for the success of PCR as a DNA-building tool lies in its simplicity. At high temperature (about 95 °C), the double-stranded target DNA denatures — unwinds into two single strands. Synthetic sequences of single-stranded DNA, known as primers, are used to bracket the region of the chain to be amplified: one primer is complementary to one DNA strand (at the start of the target region), with the second primer being complementary to the other DNA strand (at the end of the target region). The primers are annealed to the single strands when the local temperature is reduced to between 50 and 65 °C. This is followed by 'extension', at a slightly higher temperature (about 72 °C), in which a complementary strand develops from each primer by the catalytic action of a DNA polymerase enzyme, in the presence of free deoxynucleoside triphosphates. This three-step process constitutes one PCR cycle, and if repeated n times will yield 2^n copies of the original DNA strand.

Conventional instruments for performing PCR (known as thermal cyclers) are conceptually simple but possess a number of technical frailties that limit the speed and efficiency of amplification. A fundamental requirement for efficient amplification is rapid heat transfer. It is desirable to have a system with a low heat capacity that can transfer heat quickly to the sample on heating, and quickly away when cooling. Most conventional thermal cyclers have large thermal masses, resulting in high power requirements and slow heating and cooling rates. Consequently, total reaction times are typically in excess of 90 minutes.

As the denaturation and annealing steps occur as soon as the correct temperature is reached, and extension is limited only by the processing power of the polymerase enzyme (between 50 and 100 bases per second), total reaction times can be drastically shortened if the thermal mass of the instrument is reduced. Microfabricated PCR systems have been developed with this idea in mind: although diverse in structure, all rely on the reduction of thermal mass to facilitate rapid heating and cooling, and afford reaction times as short as a few minutes.

The normal approach to instrument miniaturization involves the direct downsizing of system dimensions to reduce thermal masses. An alternative is continuous-flow amplification: instead of heating and cooling a static sample to effect PCR, the sample is moved continuously through multiple reaction zones held at specific temperatures. Originally described by Kopp *et al.*[6], this approach allows ultrafast reaction times, because the small-volume fluid elements can be heated or cooled to the required temperature within 100 milliseconds.

Obeid *et al.*[5] have used this concept to demonstrate the functional integration of reverse transcription and PCR within a single microchip. The efficacy of the approach stems from the performance gains afforded through miniaturization and the operational flexibility inherent in the fluidic design. The planar glass chip is fabricated using standard photolithographic and wet-etching techniques and contains a single-channel

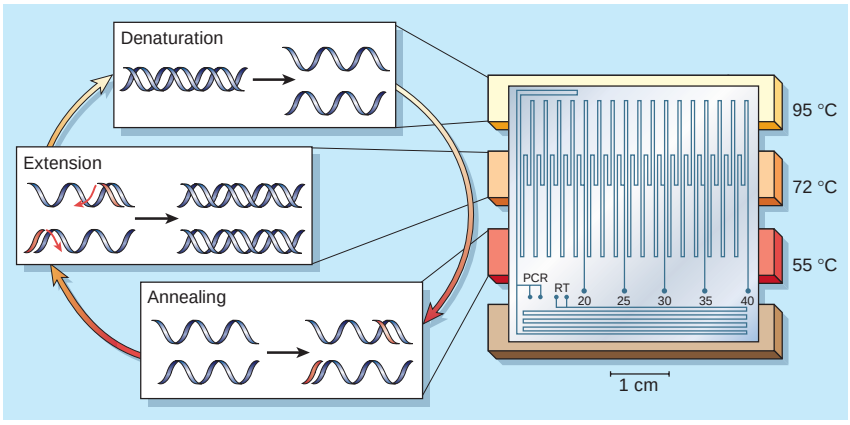

Figure 1 **DNA and chips. Obeid *et al.*[5] have built a microscale device in which DNA can be amplified quickly using the polymerase chain reaction (PCR). When a sample is added at either of the PCR inputs it flows over heating blocks whose temperatures are set to induce the three steps of PCR consecutively: denaturation, or unwinding of the DNA strands; annealing, in which primers are attached to the separated strands; and finally extension of the primers into complete DNA strands, to produce two copies of the original DNA strand. Samples can be extracted from the chip after between 20 and 40 PCR cycles, at the points indicated. A further advantage of this design is the channel for reverse transcription (RT), in which RNA samples can be transcribed into DNA before entering the PCR region of the chip for amplification.**

network defining four zones, one for reverse transcription and three for PCR (Fig. 1). Zone temperatures are controlled by simply placing the entire chip over four temperature-controlled heating blocks. Five outlets for product collection are located along the channel, so the product can be analysed after 20, 25, 30, 35 and 40 PCR cycles. Using their device, the authors demonstrate efficient amplification of DNA after 20 cycles in times as short as 5 minutes. Furthermore, by periodically injecting small samples (2 μl) into the system, separated by water and air plugs, simultaneous amplification of multiple samples can be performed in continuous flow without cross-contamination.

The benefit of continuous-flow amplification is further demonstrated by this device's ability to perform reverse transcription of RNA into DNA before PCR amplification — a process widely used for the quantification of messenger RNA levels. Reverse transcription is performed within a serpentine microchannel that, downstream, intersects the PCR channel, and subsequently proceeds through the heating zones. Integration of the two processes within a monolithic device is often problematic as reverse-transcription components at high concentration can interfere with the PCR. The authors tackle this problem by reducing the flow rate at which reverse transcription is performed, so that, at the intersection of the reverse-transcription and PCR channels, the reverse-transcription mixture constitutes about 10% of the PCR volume. With this approach, high-throughput reverse-transcription–PCR (of 0.7-μl volumes) is achieved in short times and without nonspecific amplification.

The work described by Obeid and colleagues demonstrates the true integration of biologically relevant processes within a monolithic device. Importantly, continuous-flow operation offers a direct route to automated sample introduction, mixing and reaction, and thus the possibility of high-throughput sequence analysis in many practical applications. ■

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Climate change

The earlier bird

Since 1909, researchers have been catching and marking migrating birds that stop over on the island of Helgoland in the southeastern North Sea. These birds breed in Scandinavia and spend the winter in either continental Europe (short-distance migrants) or Africa (long-distance migrants). The methods of trapping — one type of apparatus is shown in the photograph opposite — have not changed since 1960. Moreover, the data cover around two dozen species, and describe the mean time of migration for all trapped individuals, not just extremes in the form of first arrivals. All of this makes the Helgoland data sets some of the best available with which to study the timing of bird migration.

Ommo and Kathrin Hüppop have now analysed these data sets, with remarkable results (*Proc. R. Soc. Lond. B* **270,** 233–240; 2003). They find that all 23 migratory bird species for which sufficient data are available pass by Helgoland on their way to Scandinavia earlier now — by two to twelve days — than 40 years ago. There is a clear division between short-distance migrants, whose mean time of passing correlates well with local temperatures, and long-distance migrants, for which increases in the NAO index (a measure of the air pressure over the North Atlantic Ocean) give a much better explanation for the earlier time of passage.

Whether a migrating bird actually lands on a small island while passing over it depends on many factors. Sudden changes in weather play a large part. So it is important to analyse large data sets to disentangle general patterns from such isolated examples. The changes in the timing of migration are apparently strong enough to become evident.

These changes, in particular the earlier passage of the long-distance migrants, raise questions about the control of spring migration. There are three main hypotheses that might explain their earlier passage. First, the moment of leaving Africa has not changed, but refuelling in continental Europe proceeds more quickly, because more food — in the form of insects — is available earlier. (Increases in the NAO index generally indicate favourable spring conditions in Europe.) Second, if the weather in Africa is also correlated with the NAO index, then the birds might leave earlier because the seasons there also change earlier. Third, the weather in

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Africa has not changed, but natural selection has altered the 'trigger values' for starting migration. Each hypothesis, if true, would mark an exciting break with existing knowledge, and I eagerly await further results on changes in spring migration. **Arie J. van Noordwijk**

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