text of ongoing efforts at vaccine development, in which vaccine preparations may need to be aimed at individual sequence subtypes[8], it is clear that diversity surveys must include measures to identify mosaic viruses. Finally, it is important to realize that our present survey almost certainly underestimates the frequency of co- or superinfections, since recombinants of divergent viruses from the same sequence subtype are much more difficult to detect.

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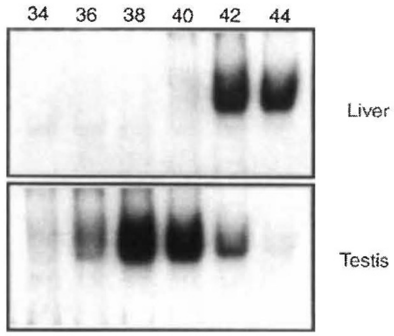
Altered stress response in testis

SIR — Heat-shock factor (HSF), a transcriptional regulator protein exhibiting heat-activatable DNA binding, mediates the stress-induced expression of eukaryotic heat-shock protein genes (for review see ref. 1). The temperature set-point for HSF activation varies between species, but it is unknown whether this set-point is identical in all cells of a single organism. A unique feature of male gonads of many species is their location outside the main body cavity, so that testis cells have a significantly lower growth temperature relative to cells of other tissues[2]. In the mouse, testis temperature is tightly regulated at 30 °C, 7 °C lower than the body cavity temperature at which tissues such as liver are maintained. Therefore, we sought to determine whether the temperature profile of HSF activation in mouse testis cells is identical to that in liver cells, or whether it is altered in a way that is consistent with the lower growth temperature of this cell type.

Gel-shift analysis demonstrates that HSF DNA binding in liver cells is induced by treatment at temperatures of 42 °C and above (see figure), a temperature profile similar to that observed for mammalian cell lines[3]. In contrast, testis cells induce HSF DNA-binding activity at significantly lower temperatures, exhibiting low levels at 36 °C, high levels at 38 and 40 °C, and diminishing levels at 42 and 44 °C. Gel-shift analysis in conjunction with specific polyclonal antibodies demonstrates that the HSF DNA-binding activity induced in testis cells by incubation at 38 °C, like that found in 42 °C-treated mouse and human cell lines[4,5], is composed of HSF1 (data not shown).



Temperature profiles of HSF1 activation in mouse liver and testis cells. Primary liver and testis cells from adult C3HF/SED strain mice were incubated in culture medium at the temperatures indicated for 60 min, and then extracts prepared from these cells were subjected to gel mobility shift assay as described[4]. Primary liver and testis cells were prepared by enzymatic dissociation of minced liver and decapsulated testes of 6 mice in DMEM containing 1 mM sodium pyruvate, 6 mM sodium L-lactate, 0.5 mg ml^{-1} collagenase and 0.25 mg ml^{-1} trypsin, at 32°C (testes) and 37 °C (liver) for 30 min with gentle inversion mixing. Dissociated cells were collected by centrifugation at 500*g* for 3 min. After two washes with DMEM, cells were resuspended in 12 ml DMEM (containing sodium pyruvate and sodium L-lactate), and 1-ml aliquots incubated at indicated temperatures for 60 min. Cells were washed with ice-cold PBS, quick-frozen in a dry ice/ethanol bath, and whole-cell extracts prepared for gel-shift analysis[4].

These results show that mouse testis cells activate HSF1 at a significantly lower temperature than liver cells, demonstrating that the temperature setpoint for HSF activation does not have a fixed value in a given species, and can vary in a cell-type-dependent manner. These findings are consistent with previous studies suggesting that HSF is not activated in response to absolute temperature experienced by the cell, but rather in response to a change in temperature[6,7]. In fact, our results suggest a tight coupling between cellular growth temperature and HSF activation temperature. In spite of their 7 °C difference in growth temperatures, HSF activation in both mouse and liver cells occurs at temperatures 4 – 5 °C above their respective growth temperatures.

What difference exists between cells which could modulate the HSF set-point? Heat-induced protein denaturation has been proposed to be the signal that triggers HSF activation[8]. Therefore, one explanation is that HSF activation temperature is directly tied to the protein thermal denaturation profile characteristic of each cell, particularly the earliest-occurring thermal transitions[9]. We propose that the lowered HSF activation temperature in testis cells may be related to a reduced thermal stability of one or more proteins expressed in these cells. Likely candidates are testis-specific proteins, whose thermal denaturation profiles could have been shifted during evolutionary adaptation of testis cells to their lower growth temperature.

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Chicken and egg

SIR — I was surprised to read in your 125th anniversary issue[1] the statement "The microwave background radiation, which fills even the corners of the Universe, would psychologically have been more compelling evidence for the Big Bang if it had been predicted before its discovery in 1965."

Indeed, microwave background radiation was predicted before its discovery, so much earlier that it must have escaped the notice of the editor and many other scientists as well. In fact, as far as I know, the first calculation of the background radiation temperature reported appeared in a brief *Nature* article[2]. The magnitude of the temperature reported at that time was ~5 K. The details of the calculations were published a short time later in several places.

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