tions of methane; gas hydrates are ubiquitous under the floor of the lake, as shown by seismic profiling[5]. The small dependence of the near-vent community on photosynthesis suggests that vents of this kind could have been important in the nascence of the unique faunistic complex of Lake Baikal consisting of 1,500 endemic species: vents could have many times served as refuges under unfavourable climates, and sources of species radiation under more favourable ones during the 20-million-year-long history of the lake. Communities of organisms built of ancient carbon are not uncommon in a marine ecosystem[6], but this is the first time they have been found in a freshwater ecosystem.

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Another *obese* gene function

SIR — The cloning of the *obese (ob)* gene[1] is indeed a breakthrough in obesity research. As Rink highlighted in News and Views[2], the evidence strongly suggests that the normal *ob* protein is a previously undescribed hormone which regulates satiety. I would like to comment on another potential function of the *ob* gene.

When allowed free access to food, *ob/ob* mice eat more than normal *ob/–* mice and develop obesity. However, the lean body mass of *ob/ob* mice is lower than *ob/–* mice, and shows characteristics of stunted animals[3]. Furthermore, food restriction to the level of the normal *ob/–* mouse does not reduce adiposity of the *ob/ob* mouse, but it only causes a further decrease in lean body mass with little change in fat/lean body ratio compared withthe *ob/ob* mouse fed without food restriction[4]. The same results are observed in the *fa/fa* rat, another genetic model of obesity caused by a single recessive gene[5]. The *fa/fa* rat is thought to have a homologous defect to the *db/db* mouse[6], which may have a defective receptor for the *ob* protein[2].

These effects of the *ob* gene cannot be explained by suppression of appetite and subsequent reduction of food intake. Rather, the observations indicate that the *ob* protein regulates the energy partition between fat deposits and the lean body by some mechanism(s) not secondary to the effect on food intake, and that a defect in *ob* gene function causes an increased energy flow toward fat accumulation even at the expense of lean body growth.

In humans, uncommon obesity caused by a single gene has been reported. On the other hand, humans generally show increased lean body mass when the degree of obesity increases; and the treatment of obesity by energy restriction effectively reduces body fat without reducing lean body mass. Thus, it will be particularly interesting to determine the role of the human homologue of the mouse *ob* gene in human obesity. The discovery of the *ob* gene sequence will soon lead to the answer to this question. Even if this gene has little relevance to most obesity in humans, the potential use of the *ob* protein for the treatment of obesity will remain.

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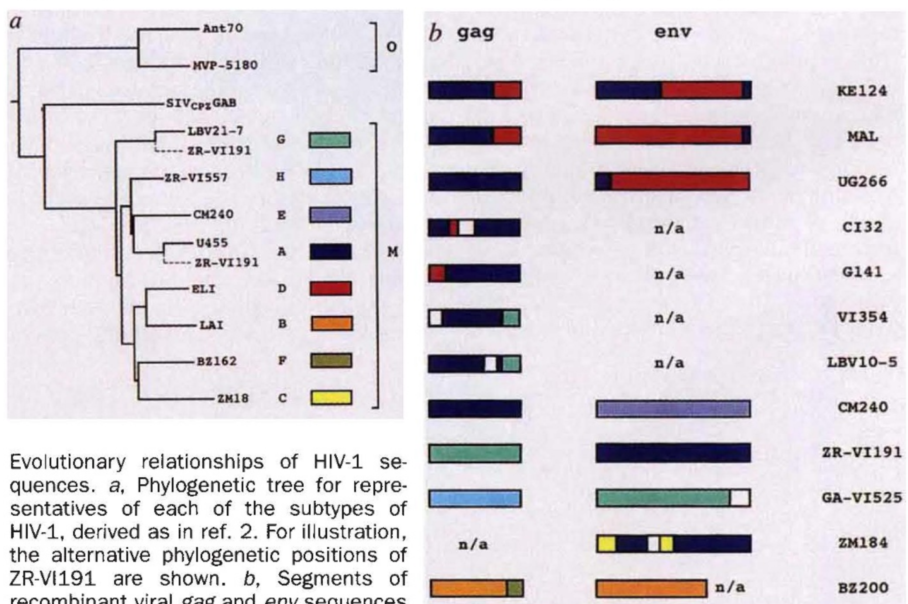
Recombination in HIV-1

SIR — Globally circulating strains of human immunodeficiency virus type 1 (HIV-1) exhibit extreme genetic diversity[1–5]. Phylogenetic analyses (*a* in the figure) have revealed two distinct 'groups' (M and O), and numerous 'sequence subtypes' within the major group M. Even though retroviruses (such as HIV-1) are highly recombinogenic[6], recombination among viruses from different subtypes has not been considered to be a significant source of new variation in HIV-1 because evidence for coinfection with multiple divergent HIV-1 strains has remained rare[7]. Here we report an extensive analysis of published HIV-1 sequences which reveals a surprisingly large number of apparently recombinant viruses. This finding has immediate consequences for our understanding of HIV-1 pathogenesis and for vaccine development[8], and of course implies that coinfection with divergent HIV-1 strains is not as rare as previously thought.

Recombination can be detected when different genes, or different regions within the same gene, are placed by phylogenetic analysis into different sequence subtypes. A single HIV-1 isolate (MAL) has long been suspected to be recombinant, and we have recently described[9] a detailed analysis of this viral genome in which the crossover points were localized. Here we have applied the same techniques to examine all HIV-1 isolates for which near-full-length *gag* or *env* sequences have been



Evolutionary relationships of HIV-1 sequences. *a*, Phylogenetic tree for representatives of each of the subtypes of HIV-1, derived as in ref. 2. For illustration, the alternative phylogenetic positions of ZR-VI191 are shown. *b*, Segments of recombinant viral *gag* and *env* sequences belonging to different subtypes; colour coding is as in *a*. Some segments (in white) cannot be designated as belonging to any currently known subtype. Localization of the breakpoints between regions of differing phylogenetic affinity is described in the table.

deposited in the database. Phylogenetic trees were constructed using various regions of the *gag* and *env* sequences: putative recombinants were identified as those sequences falling in significantly discordant positions (as assessed by bootstrapping) in different trees, and these sequences were subjected to further investigation.

Breakpoints between genomic regions with different phylogenetic histories were localized by a method adapted from ref. 10. Among 114 viruses analysed, at least 10 appear to be recombinants of sequences from different group M subtypes (*b* in the figure). In many cases recombination breakpoints were found within genes (see table), and the recombination events appear to have involved multiple crossovers, as seen (under laboratory conditions) for other retroviruses[6]. For most of these examples, the probabilities of observing (by chance) these discordant phylogenetic positions for different genes (summarized in *b* of the figure), or the nonrandom distribution within genes of sites supporting alternative phylogen-etic positions (see table), are very low. However, for completeness, we have also included two examples which are not, as yet, definitive. LBV10-5 falls as an outlier to subtype A if the entire *gag* sequence is analysed, in a position similar to other viruses (for example KE124) with mosaic *gag* genes. The 3′ end of the LBV10-5 sequence appears to be subtype G, but the test yields $0.05 < P < 0.10$; this may be because this 3′ region is fairly short, and sequencing the region downstream of *gag* could resolve this. CM240, as well as a number of other closely related viruses from Thailand, have been placed[3] in *gag* subtype A, but classified separately[4] as (the sole representatives of) *env* subtype E, for which no *gag* equivalent is yet known. CM240 is probably recombinant as it is relatively much more distant from subtype A viruses in *env* than in *gag*, but it is conceivable that this could result (without recombination) from different evolutionary rates in different regions of the genome.

Thus all eight subtypes of HIV-1 group M so far described appear to have been involved in recombination events, but no sequences were found that were hybrids of group M and group O viruses. All of the putative recombinants originated from geographic regions where multiple sequence subtypes are known to co-circulate, including central Africa, South America and southeast Asia[3-5]. Whether the frequencies of the various mosaic genomes are representative of the prevalence of recombinants at large awaits more systematic study. Future analyses should also aim to verify the presence of mosaic genomes *in vivo*, to exclude the possibility of tissue culture and/or PCR artefacts (all sequences available for this study were derived from cultured viruses).

The surprisingly high frequency of mosaic HIV-1 sequences in the database implies that a substantial proportion of individuals can become coinfected with HIV-1 strains belonging to different sequence subtypes, and that recombination between these genomes can occur *in vivo* to generate biologically active viruses, often with hybrid *gag* or *env* proteins. These results raise pressing questions concerning the global frequency of such recombinants, their biological significance, and their impact on vaccine design and evaluation. They prompt investigation of the circumstances under which coinfection (either by simultaneous transmission of divergent viruses, or by successive superinfection) can occur. They also point to the need for natural history studies addressing whether these hybrid HIV-1 genomes have new and significantly altered biological properties. In the con-

LOCALIZATION OF CROSSOVER EVENTS IN HIV-1 SEQUENCES

Isolate	Origin	Gene	Subtype	Region	Informative sites 1	Informative sites 2	Informative sites SIV_{CPZ}	P
KE124	Kenya	*gag*	A	1–1050	35	3	4	} 0.000
		gag	D	1083–1477	0	14	3	
		env	A	1–1065	30	4	4	} 0.000
		env	D	1096–2435	4	46	10	} 0.000
		env	A	2480–2580	7	1	1	
MAL	Zaire	*gag*	A	1–1068	34	6	4	} 0.000
		gag	D	1101–1518	4	12	1	
		env	D	1–2435	9	61	30	} 0.005
		env	A	2482–2580	5	1	0	
UG266	Uganda	*env*	A	1–199	10	1	4	} 0.000
		env	D	252–2556	9	62	11	
CI32	Cote d'Ivoire	*gag*	A	1–333	10	4	3	} 0.081
		gag	D	417–423	0	4	0	} 0.037
		gag	A	456–1477	21	7	10	
G141	Gabon	*gag*	D	1–261	1	13	5	}0.000
		gag	A	306–1459	38	7	5	
VI354	Gabon	*gag*	A	1–1154	38	9	14	} 0.020
		gag	G	1245–1465	1	5	1	
LBV10-5	Gabon	*gag*	A	1–1166	34	7	12	} 0.087
		gag	G	1209–1483	4	6	3	
ZM184	Zambia	*env*	C	1–328	0	11	0	} 0.000
		env	A	363–1053	13	2	12	} 0.003
		env	C	1068–1263	1	8	1	} 0.000
		env	A	1270–2547	33	7	13	
BZ200	Brazil	*gag*	B	1–1227	38	1	6	} 0.000
		gag	F	1253–1474	1	11	2	

Localization of intragenic crossover breakpoints between regions belonging to different subtypes in recombinant HIV-1 DNA sequences. For each sequence, phylogenetic analyses were performed using four taxa: the putative recombinant; two consensus sequences, each derived from nonrecombinant members of the subtypes seemingly involved in the recombination event; and an outgroup (SIV_{CPZ}GAB; *a* in the figure). The number of phylogenetically informative sites supporting the grouping of the recombinant sequence with each of the other three sequences are given. The regions were chosen so as to maximize the statistical significance (assessed by a heterogeneity χ^2 with 1° of freedom) of the difference in the distribution of sites supporting phylogenies 1 and 2: probability values (assessed from simulations) pertain to the comparisons bracketed. Regions of unclear ancestry within the *gag* genes of CI32 (at 456-852), VI354 (1-214) and LBV10-5 (762-1062), and the *env* gene of ZM184 (798-1021) (*b* in the figure) have been subsumed within neighbouring regions, leading to a probable underestimation of significance values. All sequences were obtained from the GenBank/EMBL/DDBJ database.

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references.

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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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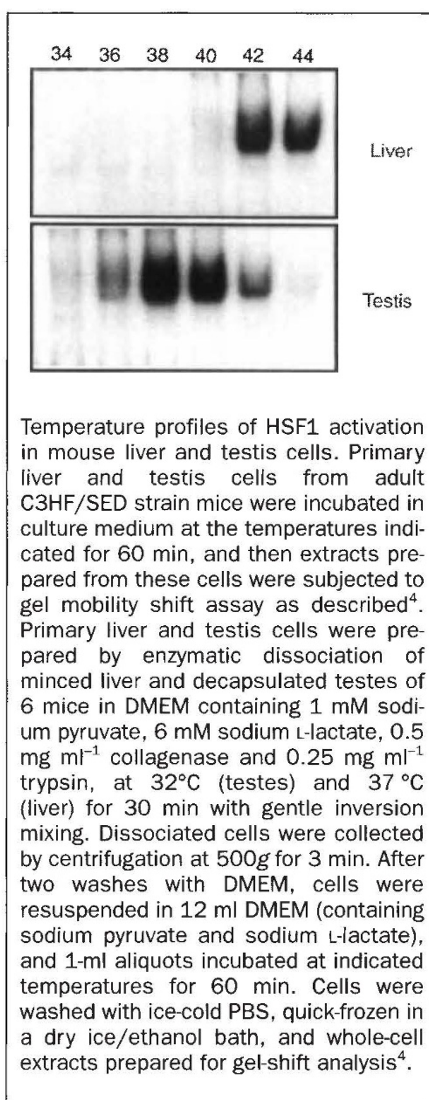
Altered stress response in testis

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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