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Cell biology

Risky immortalization by telomerase

Senescence naturally limits the proliferation of mammalian cells in culture, possibly by shortening the telomere regions at the ends of chromosomes during cell division[1,2]. In support of this idea, introducing TERT, the catalytic subunit of telomerase — the enzyme that maintains chromosome ends — into certain cell types can extend their lifespan and potentially immortalize them[3,4]. It has been proposed that treatment with exogenous TERT might be useful for cell-based therapies by allowing indefinite expansion of normal human cells without damaging their genomes[5,6]. But we show here that TERT-driven cell proliferation is not genoprotective because it is associated with activation of the c-*myc* oncogene.

Human mammary epithelial cell (HMEC) cultures normally stop dividing at 55–60 population doublings (PDs). We infected these cells with a human(h)TERT retrovirus at PD40 and maintained them until PD250 (ref. 4), then tested whether telomerase activity was essential for this immortalized phenotype by excising the hTERT retrovirus at PD150 using Cre recombinase[7]. The resulting HMEC–Cre cells were maintained for at least another 20 population doublings and we saw no decline in growth rates in either pooled cells or individual clones. We used Southern blots of genomic DNA to confirm that TERT had been removed (data not shown). To our surprise, telomerase activity remained high compared with the control parental culture, which had undetectable activity (Fig. 1a).

Ectopic expression of c-*myc* activates telomerase in HMECs[4], and hTERT is a direct transcriptional target of c-Myc[8]. To determine whether activation of c-*myc* was responsible for the telomerase activity found in our HMEC–Cre cultures, we measured the amount of Myc protein and found a two- to threefold increase in c-Myc compared with vector control cells (Fig. 1b). This is comparable to the amounts found in a culture immortalized by a *myc*-encoding retrovirus and in a breast-cancer cell line, HBL100 (Fig. 1b). The excision process did not itself cause this increase in c-Myc, as expression of c-*myc* was high before excision and also in clone 4, which still retained TERT (Fig. 1b).

We examined c-*myc* expression in HMEC–hTERT at different population doublings to determine when it was upregulated and found that it increased between 107 and 135 population doublings (Fig. 1c). Also, GADD45 protein, whose expression is repressed by Myc, decreased markedly in HMEC–hTERT at PD135 and in HMEC–Cre at PD150 compared with vector control cells and HMEC–hTERT at PD70 (Fig. 1d). These results indicate that, under standard culture conditions, extension of lifespan by telomerase selects for c-*myc* overexpression in HMECs.

Activation of the c-*myc* oncogene by overexpression, gene amplification, translocation and possibly mutation occurs in a wide variety of tumour types[9]. We have shown that, although telomerase activation extends the lifespan of HMECs, it is also associated with overexpression of c-*myc* and so is not indefinitely genoprotective (even though the chromosome number in such cells is normal; data not shown). Paradoxically, the extension of lifespan that is conferred by TERT causes c-*myc* activation, and this immortalizes cells, in part by activating TERT expression. Furthermore, in HMEC cultures, TERT expression has little, if any, immortalizing potential until the *p16* tumour-suppressor gene has been inactivated[10]. These findings indicate that the use of hTERT for expansion of normal human cells for therapeutic purposes must be approached with caution.

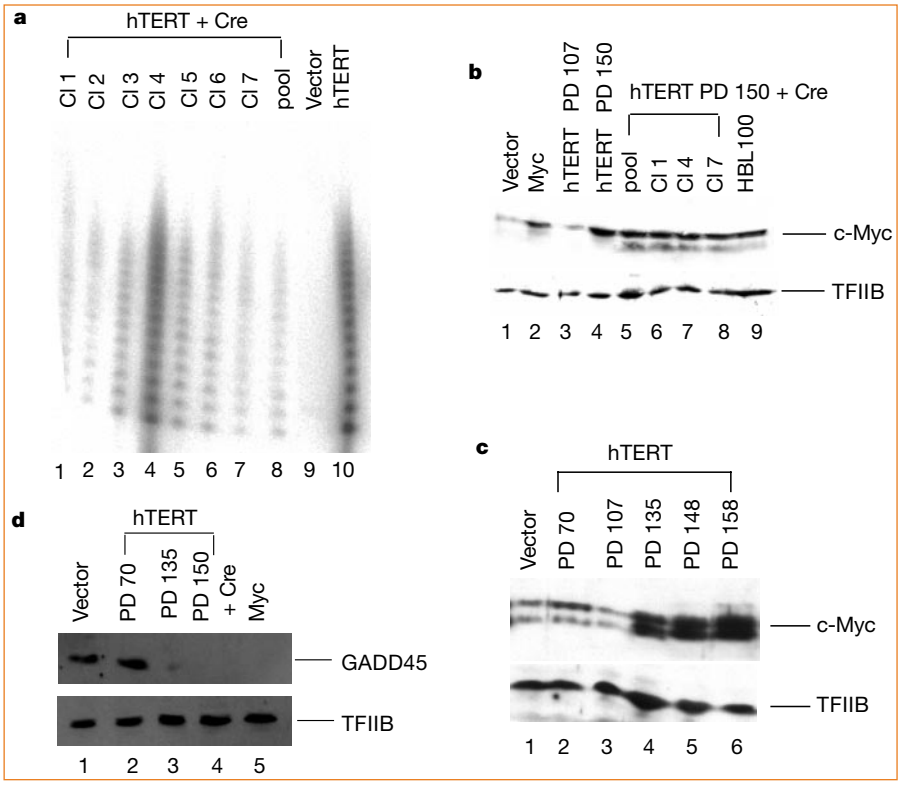

Figure 1 c-*myc* activity is increased in immortalized HMEC–hTERT cells. HMEC 184 spiral K cells were provided by M. Stampfer. All HMEC-derived cultures were maintained in complete mammary epithelium growth medium (MEGM, Clonetics) under standard conditions[11]. **a,** Telomerase activity in HMEC–Cre cells. We infected HMEC–hTERT cells at PD150 with retroviruses that direct the expression of Cre recombinase, and generated a pool (lane 8) and seven clones (Cl; lanes 1–7). The pool and individual clones, except clone 4, lost the hTERT retroviral cassette. After maintaining cultures for a further 20 population doublings (PDs), we prepared cell lysates for TRAP assays[12]. Lane 9, vector control cells at PD40; lane 10, non-excised HMEC–hTERT cells at PD150. Each lane corresponds to 10,000 cells. Results were similar in clones obtained after a second round of subcloning of Cre clone 6. **b,** Immunoblot using rabbit polyclonal anti-cMyc antibody (N-262, Santa Cruz) showing c-Myc in cell lysates from vector-infected HMECs (lane 1), cells immortalized by ectopic expression of c-Myc (lane 2), hTERT-expressing cells at PD107 (lane 3) and PD150 (lane 4), Cre-infected HMEC–hTERT pool (lane 5) and clones 1, 4 and 7 (lanes 6–8), and tumour-cell line HBL100 (lane 9). **c,** Immunoblot showing the amount of c-Myc in cell lysates from vector control cells (lane 1) and HMEC–hTERT cells at different PDs (lanes 2–6). **d,** Immunoblot using rabbit polyclonal GADD45 antibody (H-165, Santa Cruz), showing GADD45 in cell lysates from vector control cells (lane 1), hTERT-expressing cells at PD70 (lane 2) and PD135 (lane 3), Cre-infected HMEC-hTERT at PD150 (lane 4) and cells immortalized by ectopic expression of c-Myc (lane 5). In **b–d**, TFIIB protein was used to normalize loading.

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Insect perception

Do cockroaches 'know' about fluid dynamics?

Animals use their senses to extract information from the world around them, so they need to be able to gauge the physical properties of their environment in order to build up an accurate perception of it. For example, a bat needs to 'know' the velocity of sound to estimate how far away an object is, although input to a sensory system may often exploit more complicated properties than this. Here we measure the response by the wind-sensing system of the American cockroach (*Periplaneta americana*) to a complex hydrodynamic flow. We find that the insect's interneurons relay crucial information about the wind's spectral properties, which may warn it of approaching predators.

The cockroach senses minute air movements using tiny hairs on two posterior appendages called cerci[1]. It can surmise the direction of an attack and scurry away to avoid being eaten. Neural signals from the hairs converge on the terminal abdominal ganglion where the wind information is processed, and are then conveyed further by giant interneurons. Although this system has many of the properties of more complex systems, it remains simple enough to be tractable for study.

We produced random wind stimuli with defined spectral properties and measured the average firing rates of several interneurons in response to this stimulus. For a given spectral shape, the total power of the stimulus did not change the steady-state firing rates of the interneurons (Fig. 1a).

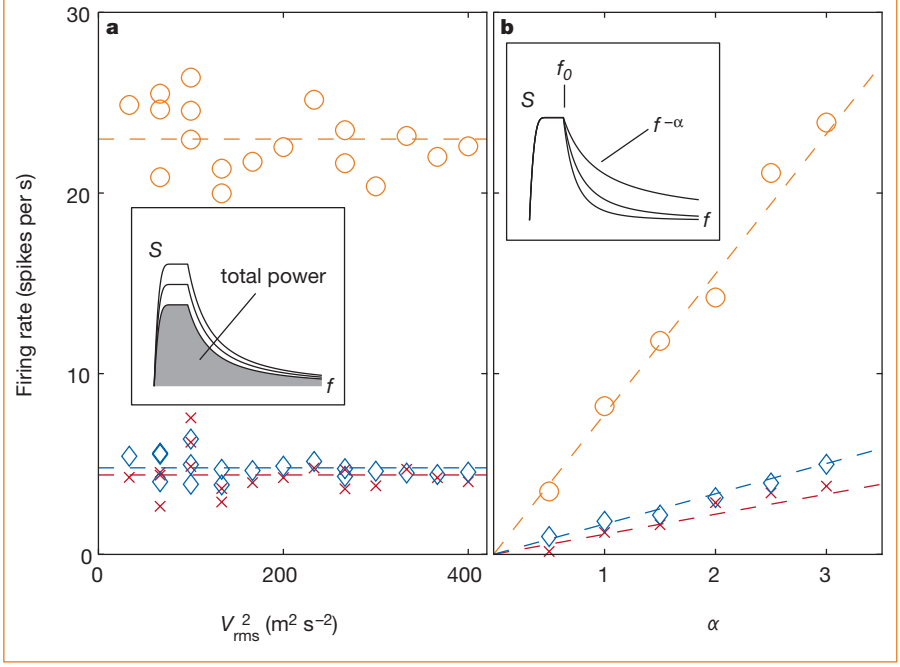

Figure 1 Average firing rate for random wind stimuli with different spectral parameters. Rates are shown for two typical interneurons (red crosses and blue diamonds) and for all interneurons together (orange circles). Inserts show spectral density, *S*, as a function of frequency, *f*, indicating how the spectral parameters were changed. The frequency f_0 was held constant for these experiments at 10 Hz. The total power, which is directly proportional to the r.m.s. of the square of the wind velocity, V^2_{rms}, and the high-frequency 'roll-off' parameter, α, were changed independently. **a,** Firing rate as a function of total power of wind spectra with $\alpha = 3$. **b,** Firing rate as a function of α shows strong dependence on the extent of the high-frequency tail.

Changing the high-frequency roll-off, on the other hand, strongly influenced the firing rates of all of the cells (Fig. 1b). Thus, exposing the system to narrow-band, low-frequency noise produces a strong cell response — that is, a high firing rate — whereas exposure to wide-band stimuli does not. In the limiting case of white noise, the firing rate is almost zero — in spite of the fact that the afferent neurons are known[2] to respond to excitations above 100 Hz. Similar effects are expected for this type of stimulus in other systems[3].

Let us now consider the typical airflow in a cockroach's environment. The Reynolds number gives an indication of the degree of turbulence[4]: given the typical size of surrounding objects (less than about 1 m in size) and the relevant wind velocities (0.1 m s^{-1}), the Reynolds number is Re $\approx 10^3$, so cockroaches live in a world that is often turbulent. Spectra with long, high-frequency tails are characteristic of turbulent airflow[5]. In contrast, the first sign of an approaching predator is slow-moving air, whose spectrum has only low frequencies: in the case of attacking toads and wasps, timescales are typically about 50 ms — corresponding to frequencies below about 20 Hz (refs 6,7). A low-frequency, narrow-bandwidth stimulus may thus be an indicator of a possible attack.

It is evident from Fig. 1 that the average firing rate of the cockroach interneurons conveys information about the spectral properties of the prevailing air movement, which change when a predator approaches. Thus, the insect's awareness of these properties and its ability to detect deviations from the norm — in the form of an excess of low-frequency winds — may help it to survive.

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Erratum

Focusing hard X-rays with old LPs

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Nature **404,** 951 (2000)

An editing error altered the intended meaning of the last two sentences of the seventh paragraph, which should read "We used PVC for focusing. As it contains a large fraction of chlorine, it provides less gain than PMMA, for example." Thus PVC is inferior to PMMA, but we used it for demonstration anyway.