



**Fig. 3** Analysis of expressions of N-myc levels in cytoplasmic RNA (isolated as described in Fig. 2A) from four neuroblastoma cell lines (SK-N-SH, SMS-KCNR, SMS-SAN, LA-N-5) treated with solvent control (C) or retinoic acid (RA) and one neuroblastoma cell line (SMS-KAN) treated with either 6  $\mu$ M 5-bromodeoxyuridine (BrdU) or 1 mM dibutyryl-cyclicAMP (cAMP). RNA was isolated from cells when they showed maximal neurite outgrowth except for SK-N-SH which does not extend neurites in response to RA but does respond by assuming a more epithelioid morphology<sup>9</sup>: SK-N-SH+RA, 2 days; SMS-KCNR+RA, 10 days; SMS-SAN+RA, 7 days; LA-N-5+RA, 13 days; SMS-KAN+BrdU, 36 days; SMS-KAN+cyclicAMP, 12 days. RNA from a human embryonic fibroblast cell lines (HEC) was used as a control. RNA was denatured in 0.44 M formaldehyde and 3  $\times$  SSC at 60 °C for 15 min, chilled on ice and twofold serial dilutions were adjusted to 20  $\times$  SSC and applied to nitrocellulose sheets. Hybridization was performed in 50% formamide, 10% dextran sulphate, 5  $\times$  SSC at 42 °C using the <sup>32</sup>P-labelled pNb-1 DNA. Washing was done in 0.2  $\times$  SSC and 0.1% SDS at 60 °C.

acquire some morphological and biochemical properties of neurones<sup>6-11</sup>. RNA was extracted from cells when they showed maximal morphological differentiation (as assessed by neurite outgrowth) and twofold serial dilutions of cytoplasmic RNA were immobilized on nitrocellulose and examined with <sup>32</sup>P-labelled pNb-1 DNA. The results of these RNA dot blots (Fig. 3) indicate that in all but one of the cell lines examined, an approximately 4–16-fold decrease in the level of N-myc expression was observed in the differentiated cells. The one cell line in which this did not occur was SK-N-SH which we found did not have detectable levels of N-myc before treatment and which has been reported not to contain amplified N-myc sequences<sup>4</sup>.

Studies on the induction of differentiation in several tumour cell lines, including teratocarcinoma<sup>14,17</sup> and myeloid leukaemia<sup>13,18</sup>, suggest that the genes required for normal growth and differentiation are not lost during tumour formation but that the regulation of these genes is altered in these tumour cells. Our experiments relate the expression of N-myc to *in vitro* morphological differentiation of human neuroblastoma cell lines and demonstrate that the level of expression of this amplified sequence is decreased in several cell lines induced to differentiate. This is consistent with previously described differential expression of proto-oncogenes during normal growth and development<sup>19</sup> and suggests N-myc is expressed early in the development of neuroectodermal tissue. Our finding that N-myc levels decrease in RA-treated neuroblastoma cells before the onset of detectable morphological differentiation, yet are not depressed in growth-arrested cultures with similar cell-cycle profiles is compatible with the hypothesized regulatory activity for the N-myc gene product<sup>20</sup>.

The presence of intracellular retinoic acid binding proteins in neuroblastoma<sup>21</sup> suggests that the prompt regulation of N-myc could be mediated by a direct effect of RA, although other mechanisms such as RA-induced increases in cyclic AMP-dependent protein kinase activity<sup>22</sup> cannot be ruled out. Since the levels of RA which induce neuroblastoma differentiation

are similar to levels that can be achieved clinically, RA itself may have therapeutic value in this disease.

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## Errata

### Accelerator mass spectrometry radiocarbon ages of amino acid extracts from Californian palaeoindian skeletons

J. L. Bada, R. Gillespie, J. A. Gowlett & R. E. M. Hedges  
*Nature* **312**, 442–444 (1984).

THIS letter was inadvertently printed before all the proof corrections had been received. The first sentence of the fifth paragraph should read 'The radiocarbon age obtained for the La Jolla Shores skeleton agrees with a previously determined 5,000–6,000 yr AMS age of this skeleton (T. W. Stafford, personal communication), whereas those obtained using the conventional radiocarbon method are much younger<sup>7</sup>.' Reference 23 should refer to the dates for the Sunnyvale skeleton, and the correct reference is: Taylor, R. E. *et al.*, *Science* **220**, 1271–1273 (1983). The fully corrected version is available in reprinted form.

### South American modern beach sand and plate tectonics

P. E. Potter  
*Nature* **311**, 645–648 (1984).

IN the title, 'South American' was incorrectly replaced by 'South African'.

### Corrigendum Iridium in Mississippi River suspended matter and Gulf of Mexico sediment

F. D. Fenner & B. J. Presley  
*Nature* **312**, 260–262 (1984).

THE value of iridium quoted for geochemical reference standard DTS-1 as 61 p.p.b. should read 0.67 p.p.b. (Gladney, E. S., Burns, C. E. & Roelandts, I. *Geostand. Newsl.* **7**, 3; 1982).