

## Variation in Epidermal Housekeeping Gene Expression in Different Pathological States

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**Using non-radioactive *in situ* hybridization we studied the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and PolyA<sup>+</sup> RNA in psoriasis and normal skin, and GAPDH in epidermis following application of a range of noxious stimuli, including ultraviolet radiation, Sellotape stripping and various irritants. In keeping with what might have been expected from previous results on cell culture of various non-epidermal cell types, expression of these putative control gene products is not constant. We suggest that the use of GAPDH, and possibly other control genes, may lead to error in interpreting experiments using Northern blotting or even RT-PCR of epidermal samples. Key words: glyceraldehyde-3-phosphate dehydrogenase; gene expression; *in situ* hybridization; keratinocyte.**

(Accepted August 25, 1999.)

Acta Derm Venereol 2000; 80: 2–3.

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Studies of gene expression play a significant role in much dermatology research. Such techniques include *in situ* hybridization, Northern blotting, immunocytochemistry and Western blotting. An important question relates to the analysis of results using these methods. What is the relevant unit of expression and therefore the denominator for comparing signal? For instance, in Northern hybridization it is common practice to report the quantity of a particular RNA species as a ratio to total RNA, or alternatively to messenger RNA or a housekeeping gene, i.e. as a ratio to a putative control. It is widely assumed that such denominators show constant expression despite good evidence in a number of cell culture systems that this assumption may be unwarranted (1–4). Our previous studies using *in situ* hybridization suggested that within epidermis the levels of expression of a wide range of genes may vary with physiological stress or disease state (5–7). In the present paper we have therefore looked at the expression patterns of a commonly used housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and PolyA<sup>+</sup> RNA (as a measure of messenger RNA) in biopsies of normal skin, psoriasis and skin exposed to irritants and ultraviolet radiation, using as the unit of analysis the individual keratinocyte.

### METHODS

Clinical samples were collected as described previously (5–7). Briefly, biopsies from involved psoriasis from 6 individuals with stable (untreated) plaque psoriasis and matched uninvolved areas were taken

and processed as described. Samples were also taken at between 8 and 48 h following application of a variety of irritants in Finn chambers to normal lower back skin including (i) dithranol at a concentration of 1 µg/µl in chloroform, (ii) sodium dodecyl sulphate (SDS) 5% in propylene glycol/ethanol, (iii) retinoic acid 0.5% in propylene glycol/ethanol (30:70), (iv) propylene glycol/ethanol (30:70), (v) chloroform, and (vi) control with no active agent. For each of these, 20 µl aliquots were absorbed onto paper discs and applied to the lower back of volunteers ( $n=8$ ) and samples were processed as described. In addition, biopsies were taken from sub-erythrogenic areas of skin at 8 h following exposure to a graded series of UVB doses using a monochromatic ultraviolet radiation source with a wavelength of  $300 \pm 5$  nm ( $n=9$ ), and in subjects following sellotape stripping at 24 h ( $n=2$ ).

Riboprobes were labelled using digoxigenin. In addition a poly-dT oligoprobe (Pharmacia, UK) as a marker of PolyA<sup>+</sup> RNA was labelled using a 3' labelling kit (Boehringer Mannheim) according to the manufacturers instructions using digoxigenin (8). A 1.3 kb KpnI/BamHI GAPDH cDNA fragment was cloned into pBSII-KS+, such that antisense transcripts were generated using T7 polymerase. Samples were hybridized and developed as described (5–7). Expression was assessed on an ordinal 10-point scale (0–5 in 0.5 increments). For the purposes of assessment the epidermis was comprised of 3 areas; basal cells, spinous cells, and "supraspinous" cells (granular cells or equivalent position in psoriasis biopsies). Data was analysed non-parametrically using Friedman analysis of variance. It was not possible to blind the assessment given the changes in histological appearance.

### RESULTS

The GAPDH probe gave an appropriately sized 1.3 kb fragment on Northern blots of primary cultured keratinocytes and HaCaT cells confirming its specificity (data not shown). Expression of GAPDH was increased in all areas of the epidermis of psoriatic plaque when compared with control normal skin (Fig. 1), with the differences highly significant for spinous and 'supraspinous' layers ( $p < 0.01$ ). A similar pattern was evident with the digoxigenin labelled poly-dT probe with increased PolyA<sup>+</sup> signal being seen in all layers of the psoriatic epidermis in comparison with control ( $p < 0.05$ ) (Fig. 2).

In skin exposed to a range of noxious stimuli there were similarities in response: UVB exposure resulted in a highly significant increases in GAPDH expression throughout the epidermis ( $p < 0.001$ ) (Fig. 3), whereas in stripped skin although there was a marked increase in GAPDH expression because of the small numbers, this difference was not significant. Following application of irritants, expression changes varied between irritants, but overall, expression of GAPDH tended to increase. Statistically significant increases in expression of GAPDH were seen following application of propylene glycol/ethanol and retinoic acid 0.5% in propylene glycol/ethanol ( $p < 0.05$ ).

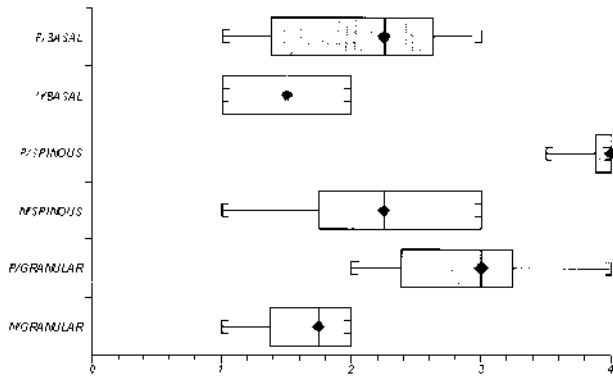


Fig. 1. Expression of digoxigenin labelled glyceraldehyde-3-phosphate dehydrogenase mRNA between psoriatic plaque and uninvolved skin from psoriasis patients. Data are shown as medians with ranges. Differences for granular and spinous layers are statistically significant ( $p < 0.01$ ). (P/BASAL: psoriatic basal layer; N/BASAL: normal basal layer, etc.).

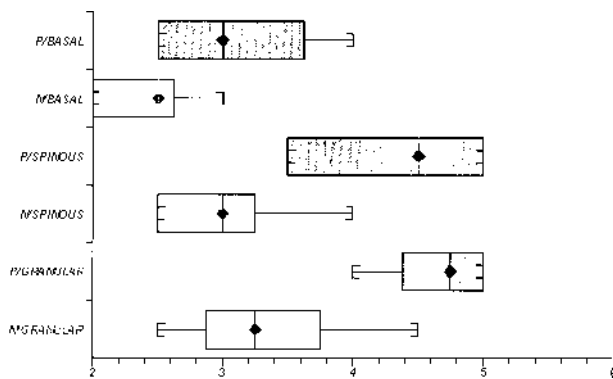


Fig. 2. Expression of digoxigenin labelled PolyA<sup>+</sup> RNA between psoriatic plaque and uninvolved skin from psoriasis. Differences between normal and psoriatic samples are all highly significant ( $p < 0.01$ ). (P/BASAL: psoriatic basal layer; N/BASAL: normal basal layer, etc.).

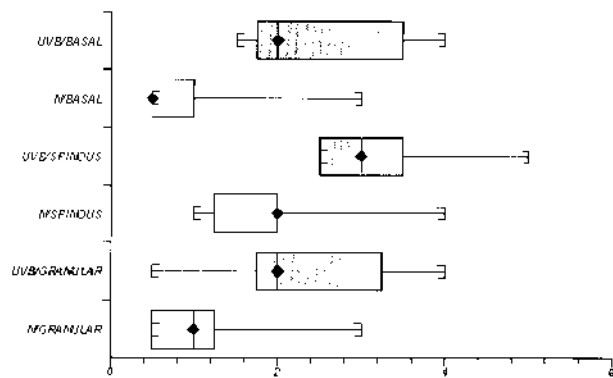


Fig. 3. Expression of digoxigenin labelled glyceraldehyde-3-phosphate dehydrogenase mRNA between controls and skin exposed to a sub-erythral dose of UVB. All differences between the irradiated and non-irradiated are highly significant ( $p < 0.001$ ). (UVB/BASAL: basal cells from irradiated samples; N/BASAL: basal cells from unirradiated samples, etc.).

## DISCUSSION

These results on *in vivo* epidermis are in keeping with previous studies on a number of non-cutaneous cell cultures (1–4). Although our analysis was only semi-quantitative, we have seen similar results using S<sup>35</sup>-labelled probes (unpublished), and the consistency of the results suggest they are not artefactual. Our conclusion is that when comparing expression of various gene products using either Northern or even RT-PCR, reference to a housekeeping gene, such as GAPDH, may lead to misinterpretation of the results. For instance, if changes in gene expression are tracked following application of retinoids, without a reliable control, interpretation of expression differences will be problematic, as it will not be clear whether the gene product under investigation, or control, is responsible for any change in ratio. The advantage of *in situ* hybridization is of course that expression is implicitly normalized to the individual cell rather than per unit RNA, although as a technique it does not lend itself readily to quantification. This leaves the question unanswered of whether it is the absolute frequency, or the relative frequency of a gene product within a cell that is the most biologically relevant. Although use of total RNA, which includes mainly ribosomal RNA, might be thought of an alternative, this is not without similar problems, as levels may also vary (1–4). If changes in gene expression studies using Northern blotting or RT-PCR are modest we suggest that they may not always be amenable to correct interpretation; similarly genuine changes may be missed without consideration of independent changes in “control” gene expression.

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