

## Expression of Transglutaminase I in Human Anagen Hair Follicles

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**The expression of the transglutaminase I in human anagen hair follicles was studied by an immunohistochemical staining. In the bulbar and suprabulbar portions of anagen hair follicles, transglutaminase I was detected on the hair cuticle and the three layers of the inner root sheath. Subsequently, the positive staining became translocated to the inner site of the outer root sheath in the middle part of the hair follicle. In the upper portion of the hair follicle transglutaminase I was detected in the internal part of the outer root sheath and the surface epidermis. Therefore, it was suggested that the expression of transglutaminase I might be closely associated with the terminal keratinization in the anagen hair follicles. Key words: programmed cell death; innermost cell layer; terminal differentiation.**

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The formation of cornified envelopes is one of the major events in the terminally differentiating epidermal keratinocyte (1). The process requires the enzyme transglutaminase (TGase), which catalyzes Ca<sup>++</sup>-dependent cross-linking of pre-existing precursor proteins via  $\gamma$ -glutamyl- $\epsilon$ -lysine isopeptide bonds (2, 3). Three forms of TGase have been identified in cell homogenates of cultured human keratinocytes. One soluble form is thought to be involved in the embedding of cytokeratin macrofibrils in the cytosol matrix of the corneocyte, whereas a particulate entity is involved in the assembly of a cornified envelope (4–6). Harding & Rogers (7) biochemically demonstrated that the predominant cross-link was the  $\epsilon$ -( $\gamma$ -glutamyl) lysine bond in the citrulline-containing proteins of the hair medulla and inner root sheath cells of guinea pig hair follicles. Further, incubating isolated mouse hair follicles with dansylcadaverine showed TGase-mediated incorporation into endogenous substrates (8). Using immunogold-silver techniques, Parenteau et al. (9) showed that monoclonal antibody, B.C1 (4), to the human TGase I binds not only to the spinous and granular layers of epidermis, but also to the inner and outer root sheaths of rat hair follicles. In the present study we studied the localization of TGase I on human anagen hair follicles by an immunohistochemical staining using the B.C1 monoclonal antibody.

### MATERIALS AND METHODS

#### Skin tissue

Human scalp skin was obtained surgically from a normal site. The specimens were embedded in Ames OCT, snap-frozen immediately in liquid nitrogen, and stored at -70°C until used. Serial 6- $\mu$ m sections were cut on a cryostat at -20°C, air-dried, and fixed in acetone just prior to the immunoassay.

#### Antibody

The B.C1 monoclonal antibody which recognized TGase I was kindly provided by Dr. R.H. Rice, University of California, Davis.

#### Immunohistochemical staining

Cryosections were incubated with a 1:640 dilution of the antibody in 0.01M phosphate-buffered saline (PBS) at pH 7.2, and then with biotinylated rabbit anti-mouse IgG antibody. They were treated with avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, Calif., USA). Finally, they were stained in a solution of 3, 3'-diaminobenzidine (0.2 mg/ml) and hydrogen peroxide in PBS.

### RESULTS

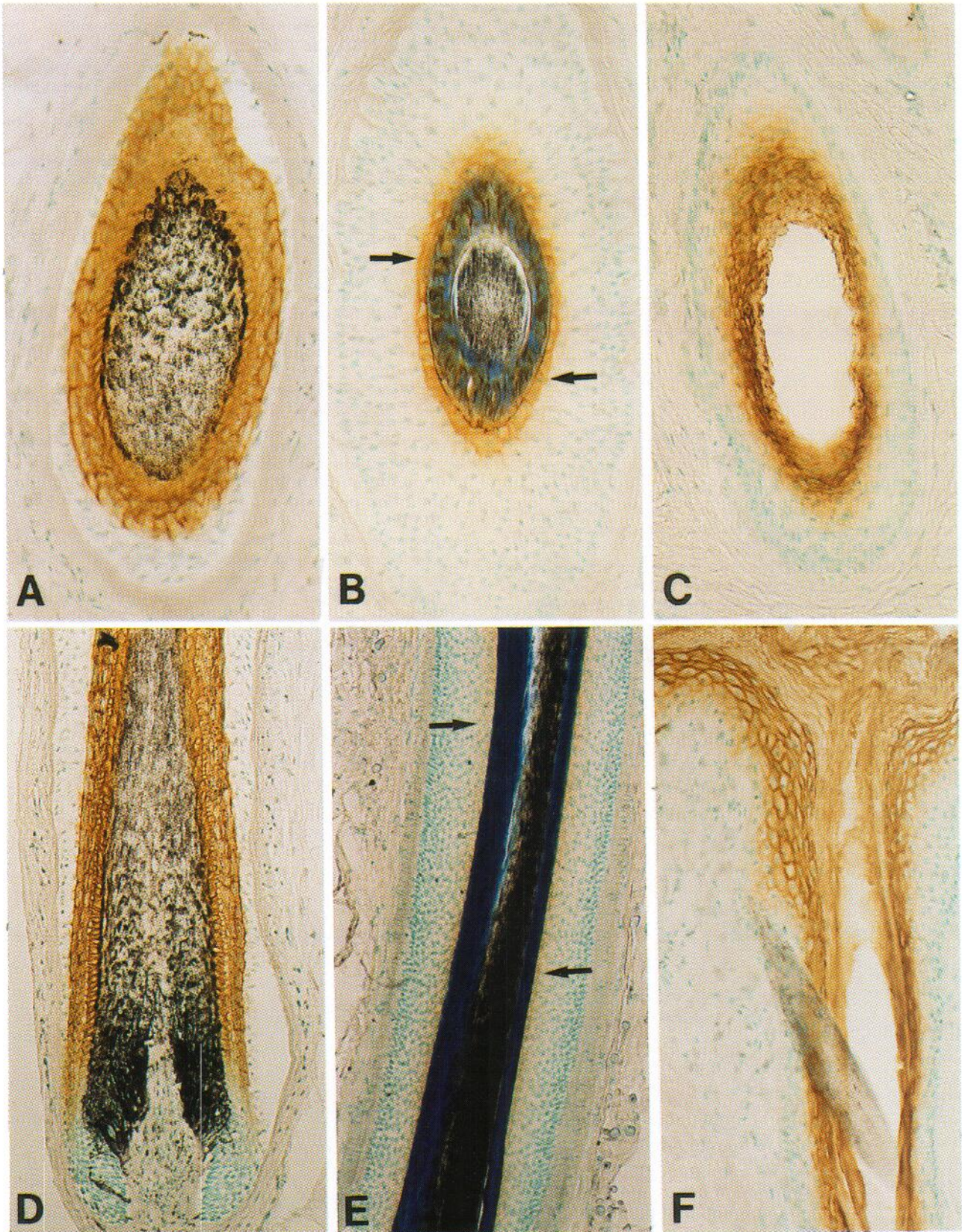
In the bulbar and suprabulbar portion of normal adult anagen hair follicles, TGase I was clearly detected on the hair cuticle and on the three layers of the inner root sheath, i.e. the inner root sheath cuticle, Huxley's layer, and Henle's layer. Especially, its strong positive staining was located on the cell membranes of those three layers (Fig. 1A, D). In the level of the proximal and middle portion of the isthmus, the positive staining of TGase I was still detectable in the keratinized area of the inner root sheath and became translocated to the inner site of the outer root sheath (Fig. 1B, E). In the region of the distal portion of the isthmus and the infundibulum, the positive staining of TGase I was observed in the internal part of the outer root sheath and surface epidermis (Fig. 1C, F).

### DISCUSSION

In the present study, we have demonstrated that TGase I was expressed in human adult anagen hair follicles. To our knowledge, there is no report on the expression of TGase I in human anagen hair follicles. Its expression appeared first in the hair cuticle and three layers of the inner root sheath, and subsequently the inner site of the outer root sheath became positive. Finally, the internal part of the outer root sheath and surface epidermis were stained in the upper portion of the hair follicle. There are several reports on the presence of TGase I in the hair follicles (8–10). Especially, Polakowska et al. (10) demonstrated that TGase I was expressed on the inner root sheath in the human fetal hair follicles. We found that it was also present on the hair cuticle and the outer root sheath in human anagen hair follicles. The presence of TGase I in the hair cuticle indicated that the marginal band which was observed in the hair cuticle at the ultrastructural level (11) might be analogous to that of epidermis in its function and assembly. From these findings, it is likely that TGase I might be activated as part of the biochemical program for the terminal differentiation of hair follicles. This expression of TGase I corresponded to the appearance of programmed cell death in the hair follicles (12).

TGase I was detected on the inner site of the outer root sheath. The innermost cell layer seems to be present at the inner site of





*Fig. 1.* Immunohistochemical localization of TGase I in human adult anagen hair follicles. In the hair bulb and suprabulbar portion the hair cuticle and three layers of the inner root sheath were positively stained (A, D). The positive staining was still detectable at the inner root sheath in the proximal and middle portion of the isthmus and became translocated to the outer root sheath. Note that the innermost cell layer (arrows) is positively stained (B, E). In the distal portion of the isthmus and infundibulum, the internal part of the outer root sheath and surface epidermis were positively stained (C, F). (A-C), transverse sections; (D-F), vertical sections.  $\times 200$ .



the outer root sheath, to contain keratohyaline granules and tonofilaments in the cytoplasm, and to become keratinized in the middle of the isthmus (13, 14). Cornified envelope precursors (involucrin, pancornulin and sciellin) were also observed in the innermost cells of the outer root sheath in the region above the point of cornification of the inner root sheath (15–17). Therefore, TGase I detected in the present study may be another marker of the innermost cells of the outer root sheath. This indicates that the innermost cells form an independent layer of the outer root sheath.

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