Association of transglutaminase-like antigen with cytoskeletal proteins

Almost simultaneously but independently two papers dealing with an unique association of transglutaminase (TGase) with that of intermediate filaments (IF) such as keratins[1] and vimentin[2], appeared. The evidence for the novel association of TGase with keratin (58 kDa) stems from biophysical and biochemical analyses of keratin filaments, which elicited a greater order of aggregation in the presence of divalent cation such as calcium. The addition of the chelator, i.e. EDTA abolished the calcium-mediated aggregation of the reconstituted keratin filaments (RKF). This implicates the exquisite specificity for calcium which functions as a cofactor for the enzyme. Addition of effectors such as cystine and histamine to the RKF resulted in a significant reduction in the TGase activity as well as kinetics of an order of aggregation. Further, Western blot obtained with polyclonal antibody against guinea pig liver TGase cross-reacts with the 58 kDa protein in the urea soluble fraction from rat vaginal epithelium. This suggests that the guinea pig liver tissue TGase and the 58 kDa keratin share a common epitope as indicated in the published work earlier[1].

In line with the above mentioned data, Trejo-Skalli *et al.*[2] mainly using immunobiological techniques, have demonstrated an association of TGase related antigen with another cytoskeleton protein widely present in the fibroblasts called vimentin. By using double label immunofluorescence antibodies to vimentin and GP2.1.2 (a monoclonal antibody raised against guinea pig liver TGase) the authors have demonstrated *in situ* that TGase-like antigen cross-react with vimentin-positive fibroblasts. After subculturing of the primary cultures, a proportional increase in the percentage of GP2.1.2 positive cells is evident. Colchicine treatment did not perturb this association, indicating that the association is very stable. Morphological observations are further supported by the findings of Western blot data where GP2.1.2 recognizes a 280 kDa protein complex of vimentin with TGase in the IF-enriched cytoskeletal preparation but does not cross-react only with vimentin per se. Further when this antibody was injected into the live dermal fibroblasts it eventually resulted in the collapse of the vimentin IF network.

What could be the physiological implication of association of TGase with cytoskeletal proteins? An analysis of the subcellular distribution of TGases may reveal clues regarding their mechanism of action. TGases encompass an array of biological functions and its diversity and specific expression in different types of cells are attributed to the physiological requirement of the cell. In recent years, there is an increasing interest in TGases because of their involvement in rendering post-translational modifications in a variety of tissues. Most notable is the cross-linking of proteins by Σ(τ-glutamyl)lysine which is a covalent cross-linkage and shows resistant to the treatment by any known enzyme so far and also by many denaturants[3,4].

Some of the other major functions of TGases include irreversible membrane stiffening of the erythrocyte, receptor-mediated endocytosis and regulation of cellular growth and its differentiation by tissue TGase[5], formation of insoluble cornified envelopes in epidermal keratinocytes by epidermal TGase[6], formation of vaginal plugs by prostrate TGase[7], formation of insoluble fibrin clots catalysed by factor XIIIa[8], and in wound healing the cross-linking of fibronectin to collagen is brought about by factor XIIIa, and nerve regenerating process observed in optic nerve by nerve TGase[9,10] and apoptotic process by tissue TGase[11,12].

Several reports are available to demonstrate the association of IF with various kinases like calcium and cAMP independent and dependent kinases[13,14]. Other cytoskeletal filamentous structures like microtubules and microfilaments also show an association with the glycolytic enzymes. This association permits localized enrichment of these enzymes for energy dependence and the cytoskeletal matrix interactions[15]. Earlier studies on HT29 or NC-N417 cells have demonstrated the association of a kinase such as PKC Σ and cytokeratin fractions[16].

A functional role of these associated TGases has not yet been ascribed. We can hypothesize that depending upon the biological signal the cell receives the corresponding TGase which may be activated to elicit its transamidating function. A key observation in our data is the demonstration of the catalytic potential for the transamidating activity of the keratin fraction by its ability to incorporate labelled ^{14}C-spermidine substrate. *In vitro* vimentin was found to serve as a substrate for TGase. In skin and in VEC the cross-linking of keratins results in the formation of NΣ(τ-glutamyl)lysine bridges which contribute to the stabilization of the filament network.

An indepth understanding of this association would not only unravel the molecular mechanism underlying the protein–protein interactions but also paves way in understanding the physiobiophysical significance of this association as their specific substrates are found among the constituent component of the IF network itself.

1. Vijayalakshmi, V. and Gupta, P. D., *Epith. Cell Biol.*, 1994, **3**, 168–174.
2. Trejo-Skalli, A. V., Velasco, P. T., Murthy, S. N. P. and Lorand, L., *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 8940–8944.
3. Vijayalakshmi, V. and Gupta, P. D., *Exp. Cell Res.*, 1994, **214**, 358–366.
4. Rice, R. H. and Green, H., *Cell*, 1977, **11**, 417–422.
5. Grasso, P. and Riechert, I. E. Jr., *Mol. Cell Endocrinol.*, 1992, **87**, 49–56.
6. Thacher, S. M. and Rice, R. H., *Cell*, 1985, **40**, 685–695.
7. Williams-Ashman, H. G., Notides, A. C., Pabalan, S. S. and Lorand, L., *Proc. Natl. Acad. Sci. USA*, 1972, **69** 2322–2325.
8. Ichinose, A., Bottenus, R. E. and Davie, E. W., *J. Biol. Chem.*, 1990, **265**, 13411–13414.
9. Eitan, S., Soloman, A., Lavie, V., Yoles, E., Hirschber, D. L., Belkin, M. and Schwartz, M., *Science*, 1994, **264**, 1764–1768.
10. Gupta, P. D., *J. Basic Appl. Biomed.*, 1994, **2**, 55.
11. Fesus, L., Davies, P. J. and Piacentini, M., *Eur. J. Cell Biol.*, 1992, **56**, 170–177.
12. Luciano, L., Gupta, P. D., Gross, S. and

Adamski, J., *Cell Death Diff.*, 1995, **2**, 259–265.
13. Osawa, S. and Hall, P. F., *Endocrinology*, 1985, **117**, 2347–2356.
14. Desser, G., Iovcheva, C., Tasheva, B. and Goldman, R., *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 2994–2998.
15. Knull, R. H. and Walsh, J. L., in *Current Topics in Cellular Regulation* (eds Stadman, E. R. and Chock, P. B.), Academic Press, New York, 1992, vol. 33, pp. 15–28.
16. Omary, M. B. Baxter, G. T., Chou, C. F., Riopel, C. L. and Lin, W. Y., *J. Cell Biol.*, 1992, **117**, 583–593.

P. D. GUPTA
V. VIJAYALAKSHMI*

Centre for Cellular and Molecular Biology,
Hyderabad 500 007, India,
**Centre for Liver Diseases,*
Owaisi Medical & Research Centre,
Deccan College of Medical Sciences,
Kanchan Bagh,
Hyderabad 500 058, India

Role of animals in the spread of human ringworm disease in Madras

Dermatophytosis in animals is an important public health problem, since this disease is frequently transmitted directly or indirectly from domestic and wild animals to man by contact[1,2]. Several workers have reported zoonotic ringworm disease of man from different parts of India[3–5]. Here we report the isolation of three strains of *Trichophyton mentagrophytes* from animals and their epidemiologic importance in human ringworm disease in Madras since such data is not available.

One lion cub, one Nilgiri langur, three lamas, and three camels in Arignar Anna Zoological Garden and 22 dogs (17 native breeds, 2 German Shepherds, 2 Doberman, 1 pomeranian), 11 cattle and 1 donkey calf in and around the city of Madras were screened. The animals were thoroughly examined for the presence of any lesion suggestive of ringworm with the help of a veterinary officer. The fur was then combed with a sterile tooth brush and then stabbed onto Sabouraud's dextrose agar plates with and without antibiotics (chloramphenicol 0.5 mg/ml, cyclohexamide 0.5 mg/ml). The plates were incubated for 6 weeks at 26°C. Identification was done based on colony morphology and microscopic characters of the fungus. Hair perforation test, urease test and pigment production in corn meal agar were also performed. Mating experiment was done or the teleomorphic identity and mating type of the isolates using the method of Padhye and Carmichael[6]. All the three strains of *T. mentagrophytes* were crossed with *Arthroderma benhamiae* RV26778(+), RV26780(–), *Arthroderma benhamiae* (African race) RV30000(+), RV30001(–), *Arthroderma vanbreuseghemii* RV27960(+), RV27961 (–) and *Arthroderma simii* RV54201(–), RV25472(+) respectively. Sterilized garden soil baited with sterilized horse and guinea pig hair was used for conducting mating experiment. The plates were incubated at 26°C for six weeks away from light. Teleomorphs and mating types were identified on the basis of the production of gymnothecia. Asci and ascospores were examined microscopically.

In the present study, we isolated three strains of *T. mentagrophytes*, one each from a lion cub and two dogs. No symptomatic lesions were observed in these animals suggesting that animals may act as carriers of dermatophytes. All the animal isolates of *T. mentagrophytes* and 6/70 clinical isolates of *T. mentagrophtyes* isolated in our previous study produced gymnothecia with *A. vanbreuseghemii* (–) mating type and was identified as *A. vanbreuseghemii* (+) mating type. The six clinical isolates which were recovered from severe cases of tinea capitis in children belonged to rural Madras. These children would have contracted the disease while playing with animals. The isolation of one strain of *T. mentagrophytes* from a lion cub suggests that wild animals also may harbour pathogenic dermatophytes. Though the number of animals screened in the present study was small, findings strongly suggest that animals may act as vectors of human ringworm disease, especially in rural areas.

1. Georg, L. K., *Trans. N.Y. Acad. Sci. Ser.*, 1956, **18**, 699.
2. Kaplan, A., *Arch. Dermatol.*, 1967, **96**, 404.
3. Gugnani, H. C., Mulay, D. N. and Murty, D. K., *Indian J. Derm. Venerol.*, 1967, **33**, 73.
4. Tewari, R. D., *Agra Univ. J. Res.*, 1962, **12**, 163.
5. Klokke, A. H. and Durairaj, P., *Sabouraudia*, 1967, **5**, 153.
6. Padhye, A. A. and Carmichael, J. W., *Sabouraudia*, 1969, **7**, 178.

S. RANGANATHAN
THANGAM MENON
S. ARUN MOZHI BALAJEE

Department of Microbiology,
Dr ALM Post Graduate Institute of Basic Medical Sciences,
Taramani, Madras 600 113, India