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SEPARATION OF INDUCTION AND EXPRESSION OF TIGHT JUNCTION FORMATION MEDIATED BY PROTEINASES

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The formation of tight junctions can be induced in the human adenocarcinoma cell line HT 29 by treatment with trypsin at 37°C. In contrast, after treatment of the cells with trypsin at low temperature (3°C), no tight junctions were observed. However, abundant formation of tight junctions occurred when cells were treated with trypsin at 3°C, washed with soybean trypsin inhibitor, and subsequently incubated at 37°C. Thus, this protocol allows for the first time the temporal separation of the induction and assembly of tight junctions.

Tight junctions are structurally specialized cell membrane domains formed at regions of contact between two cells. They commonly occur in epithelia as a continuous belt of intimate contacts and usually form the most luminal elements of the junctional complex where they constitute an efficient diffusion seal. Tight junctions are best characterized by freeze-fracturing where they appear as ridges or beaded strands on P-face and as complementary furrows on E-face [1]

The molecular structure of tight junctions is controversial. Whereas it has been proposed that the fibrils observed in freeze-fracture electron microscopy constitute intramembrane proteins [1–4], it was recently suggested that lipids constitute all essential components of tight junctions [6,7]. Little is known on the formation of tight junctions. Their assembly could be induced in different experimental systems by various agents such as proteases [8–10], vitamin A [11], incubation of the tissue in an atmosphere with a high content of CO_2 [12], and cyclic nucleotide application [13].

We have shown earlier that the formation of tight junctions can be induced in cultured HT 29 cells [10]. This cell line was originally derived from a human colon carcinoma. Repeated observations have shown that the cells have no tight junctions whatsoever. However, abundant formation of such junctions occurs after brief treatment with trypsin [10]. Even rather low concentrations of trypsin such as 25 μg/ml can induce the formation of a rather complex meshwork of junctions at 37°C after treatment for 15 min. We have now studied the effect of trypsin treatment at low temperature.

When HT 29 cells were treated with 0.05 mg trypsin/ml at 3°C for periods of 2 min–3 h, no tight junctions whatsoever were formed. Treatment under otherwise the same conditions with a high concentration of trypsin (2.5 mg/ml) produced very short junctional segments in 1.7–6.9% of the cells (Table I). However, when HT 29 cells were treated at 3°C with trypsin (0.05–2.5 mg), washed with soybean trypsin inhibitor in the cold, and subsequently incubated at 37°C for 15 min, abundant formation of junctions was observed by freeze-fracture electron microscopy (Table I, Fig. 1). Treatment with 0.05 mg trypsin/ml for 2 min

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TABLE I

INDUCTION OF TIGHT JUNCTIONS IN HT 29 CELLS

For details see Fig. 1

Treatment	Number of experiments	Number of membranes [a] observed	Number of membranes [a] with tight junctions	%
No trypsin 3 h at 3°C	2	102	0	0
0.05 mg trypsin/ml 3 h at 3°C	2	135	1	0.7
2.5 mg trypsin/ml 2 min at 3°C	5	282	5	1.7
2.5 mg trypsin/ml 3 h at 3°C	6	275	19	6.9
0.05 mg trypsin/ml 2 min at 3°C, wash [b], incubate 15 min at 37°C	4	191	74	39
0.05 mg trypsin/ml 3 h at 3°C wash [b], incubate 15 min at 37°C	1	159	92	58
2.5 mg trypsin/ml 2 min at 3°C, wash [b], incubate 15 min at 37°C	5	273	201	74
2.5 mg trypsin/ml 3 h at 3°C, wash [b], incubate 15 min at 37°C	3	191	123	64

[a] Each membrane represents a single cell.

[b] Cells were washed twice with medium containing soybean trypsin inhibitor (1 mg/ml) and subsequently incubated in medium containing the inhibitor at the same concentration.

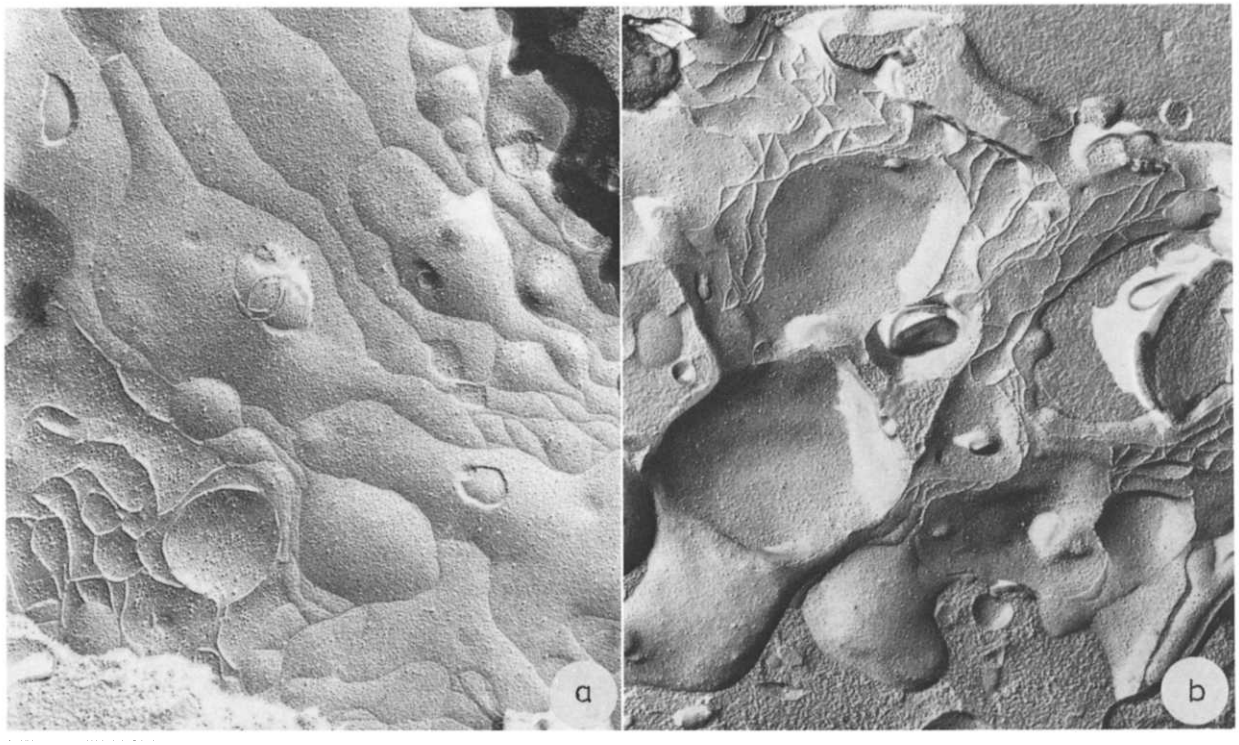

Fig. 1. Freeze-fracture electron micrographs of HT 29 cells after treatment with trypsin at 3°C and subsequent incubation without trypsin at 37°C. Cells were grown in DMEM with 10% fetal calf serum for 4–5 days. The medium was replaced by DMEM without serum and the cells were cooled to 3°C. Trypsin in DMEM was added to the final concentration indicated. The cells were kept at 3°C, washed twice in the cold with DMEM containing 1 mg of soybean trypsin inhibitor/ml and warmed to 37°C in DMEM with soybean trypsin inhibitor (1 mg/ml). After 15 min, cells were fixed with glutaraldehyde and freeze-fractured [9] Magnification: ×36000. (a) 2.5 mg trypsin/ml, 2 min at 3°C. (b) 50 μg trypsin/ml, 3 h at 3°C.

at the low temperature followed by incubation for 15 min at the high temperature was sufficient to produce complex tight junctions in 39% of the cells observed.

Control experiments showed that cold treatment without trypsin does not produce any tight junctions. It was also shown that soybean trypsin inhibitor, which was used in these experiments to neutralize trypsin, does not produce tight junctions by itself. Trypsin pretreated with soybean trypsin inhibitor did not induce tight junction formation.

These experiments indicate that a mild and brief treatment with trypsin provides a stimulus to the cells which cannot be expressed at the low temperature. However, the stimulus is remembered and can be expressed when the cells are transferred to physiological temperature. Thus, the experiment dissects the tight junction formation into two phases, namely induction and expression. This opens the way to analyze the potential involvement of various cellular subsystems (such as cytoskeletal elements, energy charge etc.) in either the induction or assembly of tight junctions. Such experiments are under way.

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