differences between the *formae speciales* were relatively small and the determinants for the host specificity could be combined or lost in individual strains.

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Cytochalasin B and taxol modulate cell surface ultrastructure in hydra

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Direct physical contacts between neighbouring cells in embryos, tissues and organs are often governed by changes in the cell surface architecture. Cytoskeleton is one of the cell organelles that regulate cell surface architecture. We have studied the role of microfilaments and microtubules in maintenance of cell surface architecture in diploblastic hydra by using drugs that specifically interact with individual cytoskeletal components. Adult hydra were exposed to 10 μM concentration of either the microfilament-disrupting agent cytochalasin B or the microtubule-stabilizing drug taxol for 1 h and cell surfaces were examined by scanning electron microscopy. It was found that changes in microfilaments and microtubules alter the cell surface in hydra although the effects of the two are quite different. The present results suggest the possibility that func-

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tional interaction between cytoskeletal components and cell surface architecture evolved early in evolution.

Keywords: Cell surface architecture, cytochalasin B, hydra, scanning electron microscopy, taxol.

CELL surface architecture is crucial for cell and tissue interactions during pattern formation. Dynamic nature of the two cytoskeletal components, microfilaments (MF) and microtubules (MT), is believed to influence surface characteristics of cells. It is well established that cellular architecture governs a wide range of cellular activities including cell proliferation, gene regulation, protein synthesis, cell adhesion and cell motility. It has been suggested that cytoarchitectural changes regulate the formation of complex multicellular tissues during development[1]. Interactions between cell surface components and the underlying cytoskeleton have been recorded in a large number of organisms and are likely to have evolved early in evolution.

The diploblastic hydra, which belongs to the basal eumetazoan phylum Cnidaria, is one of the earliest animals that exhibit a definite body plan. Hydra has been a favourite model for developmental biologists[2,3] as some of the basics of pattern formation and tissue regeneration can be studied more readily in hydra owing to its simple cellular organization. In the past 4–5 years, importance of hydra as a model in developmental biology has further increased as homologs of a large number of vertebrate development-specific genes have been detected in hydra (reviewed in ref. 4). Products of these genes require proper cellular interactions to drive morphogenesis. Cell surface and cytoskeleton play a crucial role in such interactions. It is likely, therefore, that structural and functional interactions between the cell surface and the cytoskeleton may have been established quite early in evolution. To explore this possibility, we have studied the role of MF and MT, in the maintenance of various features of the cell surface in *Pelmatohydra oligactis* by employing cytochalasin B (CB) and taxol. CB is a secondary mould metabolite that disrupts MF[5], while taxol is an alkaloid from the Pacific Yew that induces excessive stabilization of MT, thereby affecting their organization[6,7]. We have used the time-tested technique of scanning electron microscopy (SEM) that gives a three-dimensional view of the external side of the cell surface. Our results show that MF and MT regulate cellular interactions in hydra by modulating the cell surface.

CB was obtained from Sigma Chemical Company, USA while taxol was a kind gift from Drug Synthesis and Chemistry Branch, Developmental Therapeutic Program, Division of Cancer Treatment, National Cancer Institute, USA.

Clonal culture of *Pelmatohydra oligactis* was maintained in hydra medium[8] at room temperature (22–26°C). Hydra were fed on alternate days with *Daphnia* and *Cyclops*. Full-grown healthy hydra were selected, starved for 48 h before treatment and distributed in 3 groups of 20 hydra each. These were treated with CB (10 μM) or taxol (10 μM) for 1 h. Appropriate solvent (DMSO) controls were maintained.

At the end of the treatment, hydra were made to relax by treatment with 2% urethane for 30 to 40 s and processed for SEM as described earlier[9]. Hydra were fixed in 2.5% glutaraldehyde at 4°C overnight, dehydrated in graded series of ethanol and critical point dried from CO_2 in a Bio-Rad E3000 critical point drier. Hydra were mounted on stubs with double-sided adhesive tape, coated with gold in a Bio-Rad E5200 automatic sputter coater and studied with a Cambridge Stereoscan S120 scanning electron microscope. Representative areas were photographed on a 35 mm black and white negative film.

We have studied the effects of CB and taxol on surface architecture of hydra cells by SEM. Four discrete areas of hydra body were chosen for this study, namely, the tentacles, the hypostome, the body column and the stalk (Figure 1). To begin with, the effects of dimethyl sulphoxide (DMSO), which was used as a solvent for CB and taxol, were analysed. We did not notice any difference in the surface ultrastructure of cells from untreated and DMSO-treated hydra (data not shown). Therefore, surface architecture of cells from hydra treated with either CB or taxol was compared with cells from the solvent control (DMSO-treated) hydra.

A fully extended tentacle of a DMSO-treated control hydra was made up of epitheliomuscular cells with smooth surface and polygonal borders (Figure 2 *a*). In the central region of individual cells, several cnidocytes of nematocyte battery and sensory papillae were clearly seen (Figure 2 *d*). Cnidocils appeared as hair-like structures (Figure 2 *g*) and were organized into tufts. Each tuft represents a battery of nematocytes. Amongst the battery of cnidocils, sensory papilla could be identified as a light spot without hair or visible projection (Figure 2 *g*). Treatment of hydra with 10 μM CB for 1 h led to compaction of tentacles and disruption of cell–cell contacts of epitheliomuscular cells (Figure 2 *b*). Individual cells appeared more rounded and the points of contact between neighbouring cells were far less in number than in controls. Arrangement of battery of nematocysts and sensory papillae appeared to be adversely affected. Extensive membrane ruffling was observed (Figure 2 *e*, *h*). Treatment of hydra with 10 μM taxol for 1 h also led to disruption of cellular organization while extendibility of the tentacle was affected to a much lesser extent (Figure 2 *c*) than in CB-treated hydra (Figure 2 *b*). At higher magnifications, devastating effects of taxol on the epitheliomuscular cell surface became evident (Figure 2 *f, i*). Cells were irregularly shaped with a large number of stubby surface projections (Figure 2 *f*) and a highly ruffled membrane (Figure 2 *f, i*). Cnidocils and sensory papillae appeared to have lost their regular arrangement and were much fewer in number (Figure 2 *i*). Thus both CB and taxol adversely affected the surface ultrastructure of cells of the tentacles. The effects of CB and taxol were qualitatively different

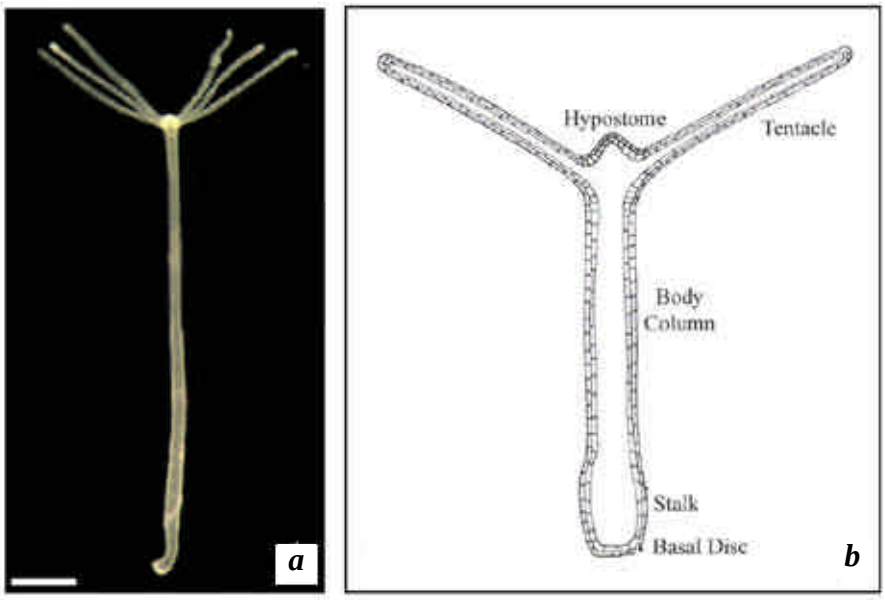

Figure 1. A live specimen of *Pelmatohydra oligactis* (***a***) and a line drawing of the same to depict its different body parts (***b***). Scale bar = 1 mm.

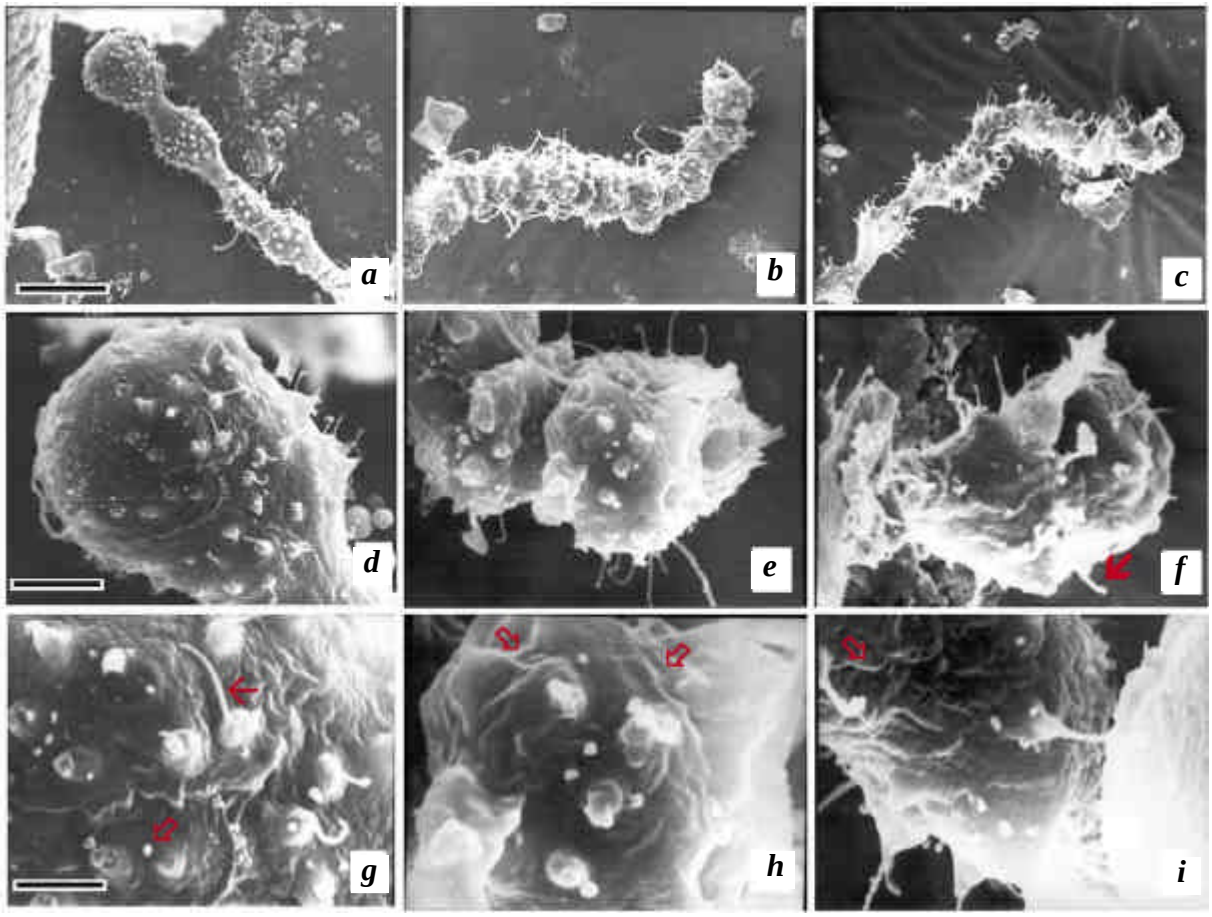

Figure 2. Scanning electron micrographs of tentacles of DMSO-treated (***a***, ***d***, ***g***), CB-treated (***b***, ***e***, ***h***) and taxol-treated (***c***, ***f***, ***i***) hydra. Cnidocils appear as hair-like structures (arrow in ***g***) while sensory papilla appears as a light spot without a visible projection (hollow arrow in ***g***). Extensive membrane ruffling occurs due to CB. Cells in taxol-treated hydra appear irregularly shaped with stubby surface projections (arrow in ***f***) and membrane ruffles (hollow arrow in ***i***). For detailed description, see text. Scale bar = 50 μm for ***a*** to ***c***, 10 μm for ***d*** to ***f*** and 5 μm for ***g*** to ***i***.

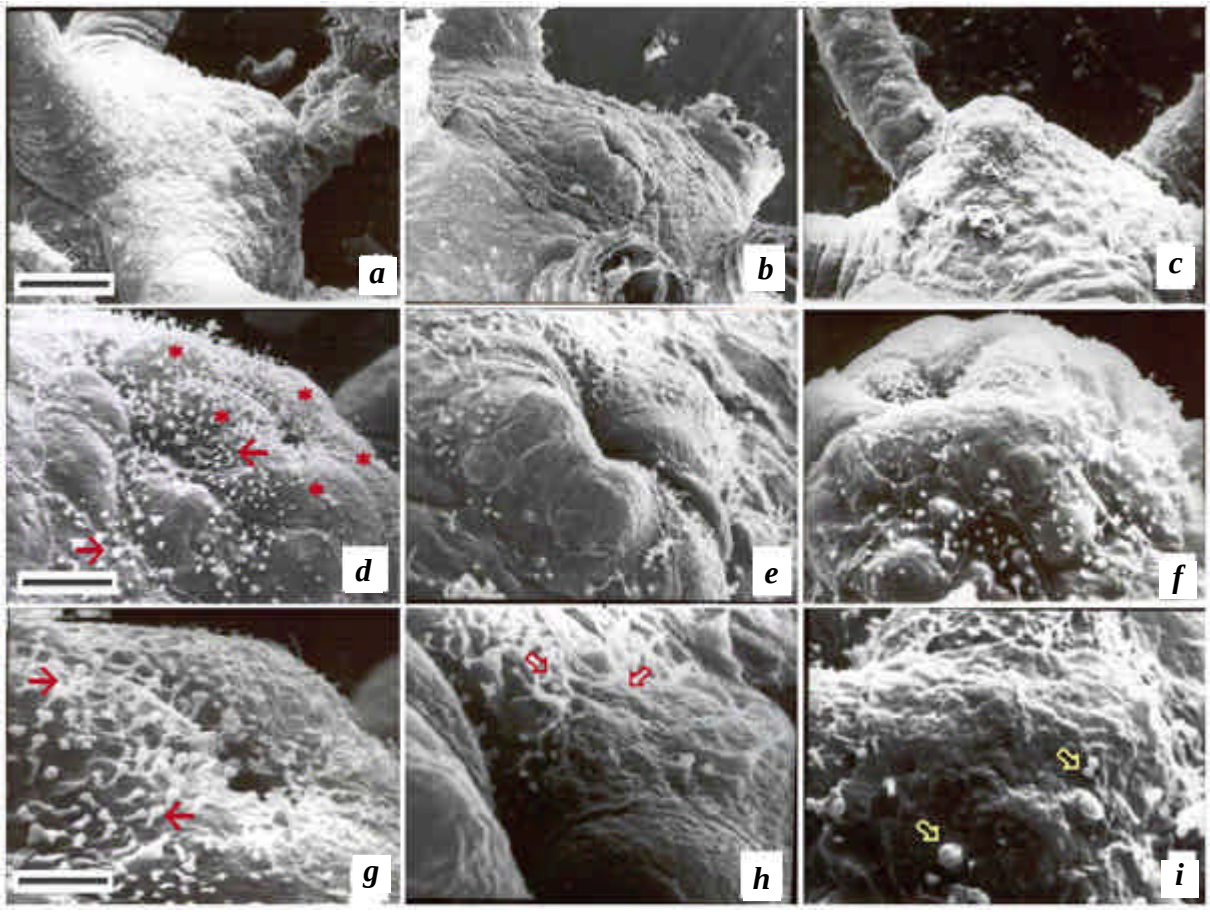

Figure 3. Scanning electron micrographs of hypostome region of DMSO-treated (***a***, ***d***, ***g***), CB-treated (***b***, ***e***, ***h***) and taxol-treated (***c***, ***f***, ***i***) hydra. In controls, rosette of outer lip cells (asterisks in ***d***) and club-shaped microvilli (arrows in ***d***, ***g***) are seen. CB induces membrane ruffling (hollow arrows in ***h***) while taxol, in addition to ruffling, induces blebbing (hollow arrows in ***i***). For detailed description, see text. Scale bar = 50 μm for ***a*** to ***c***, 10 μm for ***d*** to ***f*** and 5 μm for ***g*** to ***i***.

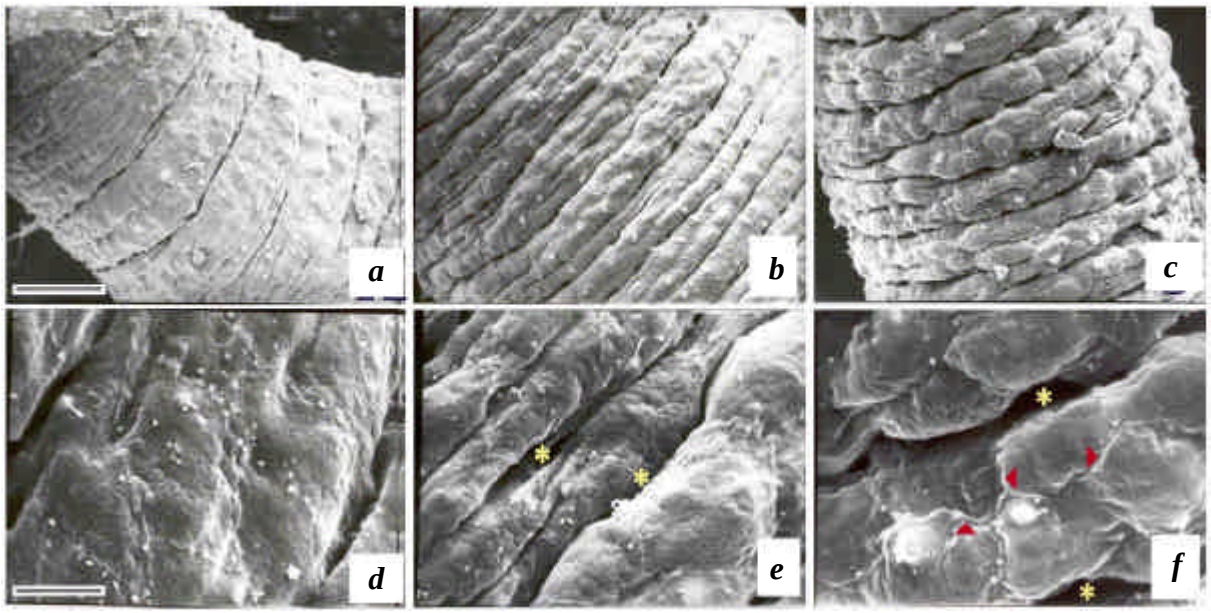

Figure 4. Scanning electron micrographs of body column of DMSO-treated (***a***, ***d***), CB-treated (***b***, ***e***) and taxol-treated (***c***, ***f***) hydra. Note appearance of big gaps between consecutive rings due to CB and taxol (asterisks in ***e*** and ***f***). Cellular outlines become more distinct in taxol-treated hydra (arrowheads in ***f***). For detailed description, see text. Scale bar = 50 μm for ***a*** to ***c*** and 10 μm for ***d*** to ***f***.

and taxol was generally more potent than CB in disrupting the cell surfaces and cell–cell interactions.

Opening of the mouth was surrounded by a rosette of outer lip cells in DMSO-treated control hydra (Figure 3 *a*). Outer lip cells along with the surrounding continuous sheet of polygonal epitheliomuscular cells exhibited a large number of club-shaped microvilli (Figure 3 *d*). These were heavily concentrated over lip cells (Figure 3 *g*). Treatment with CB resulted in complete disappearance of microvilli from the lip cells though a few persisted at the boundary between lip cells and surrounding cells (Figure 3 *b*, *e*). CB treatment also resulted in membrane ruffling (Figure 3 *h*). The effects of taxol once again appeared more drastic than those of CB. The epitheliomuscular cells seemed to have partially lost adhesive contacts with their neighbours giving an irregular appearance to the cell sheet surrounding the lip cells (Figure 3 *c*, *f*). The microvilli on lip cells appeared shorter and those on the surrounding cells were fewer in number (Figure 3 *f*). Taxol treatment induced extensive membrane ruffling and formation of blebs (Figure 3 *i*).

Body column of control hydra was made up of polygonal cells arranged in circular layers giving it an annular appearance (Figure 4 *a*). The cells were relatively smooth in appearance with a few stubby surface projections and the cell layers were tightly packed (Figure 4 *d*). The annular appearance of the body column became more pronounced after CB treatment due to bigger gaps that appeared between consecutive rings of cells (Figure 4 *b*, *e*). A few surface projections persisted after CB treatment (Figure 4 *e*). Taxol treatment resulted in disruption of cell–cell interactions as seen from disappearance of regular arrangement of cells and presence of very large gaps between consecutive rings of cells (Figure 4 *c*). Some of the cells protruded out of the layer indicating disruption of cell–cell contacts (Figure 4 *c*, *f*). Taxol treatment also led to a complete abolition of surface protrusions giving the cells a 'flattened' appearance (Figure 4 *f*). The cell boundaries became more distinct in taxol-treated hydra (Figure 4 *f*).

In control hydra, external surface of the stalk was composed of regularly and tightly arranged polygonal cells (Figure 5 *a*). The cell boundaries were quite distinct but the surface was almost totally devoid of surface projections (Figure 5 *d*). CB treatment led to a loss of tight, smooth arrangement of cells (Figure 5 *b*). A large number of extracellular projections and membrane ruffling were seen (Figure 5 *e*). At the base, the smooth appearance was replaced by an irregular outline (Figure 5 *b*). The cell boundaries too were not distinguishable (Figure 5 *e*). Taxol treatment also led to disappearance of smooth margin of the base plate (Figure 5 *c*). Surface of stalk cells was covered with tiny projections and the overall smooth cellular architecture was disrupted (Figure 5 *f*). Some of the cells exhibited a large number of small button-like blunt projections along their poorly defined margins (Figure 5 *f*).

Thus, both CB and taxol led to dramatic alterations not only in the cellular organization but also in cell surface ultrastructure. In general, the effects of taxol were more pronounced than those of CB. While the cellular organization was disrupted all over the body, cell surface ultrastructure was most drastically altered in tentacles and hypostome.

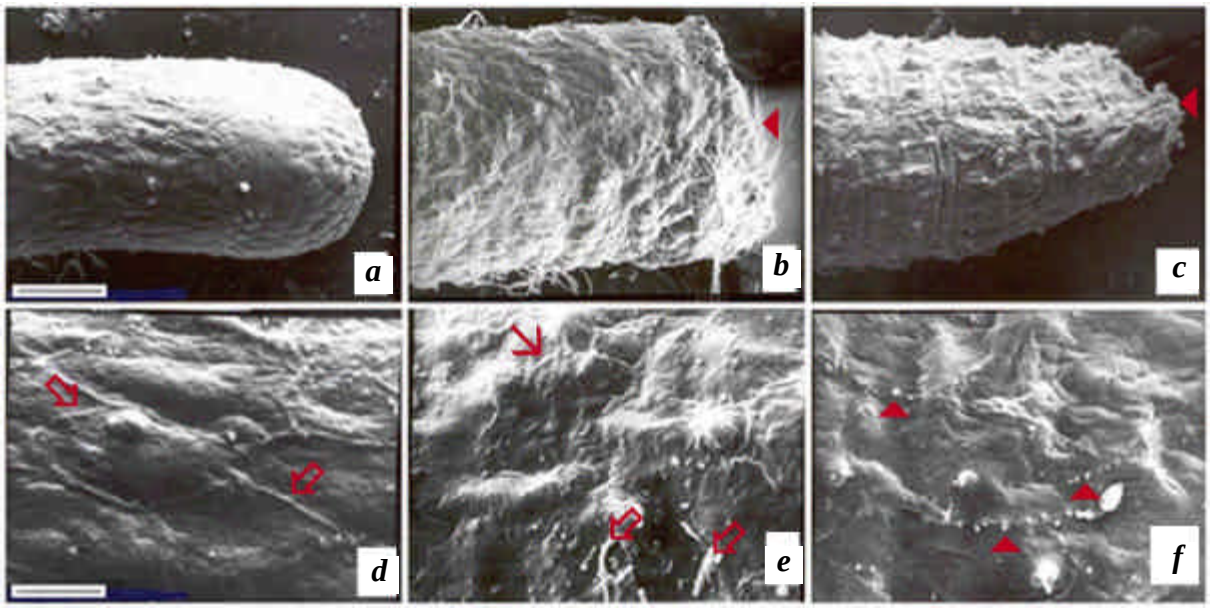

Figure 5. Scanning electron micrographs of stalk of DMSO-treated (***a***, ***d***), CB-treated (***b***, ***e***) and taxol-treated (***c***, ***f***) hydra. Note smooth cell surface with clearly defined margins in controls (hollow arrows in ***e***). CB induces extracellular projections (hollow arrows in ***e***) and membrane ruffling (arrow in ***e***) as well as irregular outline of the base (arrowhead in ***b***). Taxol indices blunt projections on ill-defined cellular margins and irregular outline of the base (arrowhead in ***c***). For detailed description, see text. Scale bar = 50 μm for ***a*** to ***c*** and 10 μm for ***d*** to ***f***.

SEM has been employed to study certain aspects of hydra biology in the past[10–15]. However, there has been no study that describes the surface architecture of the entire body of hydra. Further, to our knowledge, there has been only a single study describing the effects of cytoskeletal drugs on hydra cells[16] that was predominantly undertaken to examine the role of MF and MT in migration of nematocytes *in vitro*. The present study was undertaken to find out if a primitive animal like hydra exhibits a close interaction between the cell surface and the cytoskeleton so commonly encountered in cells of more evolved animals. To address this question, drugs that specifically interact with cytoskeletal components were used. CB and taxol are widely used to experimentally interfere with the function of MF and MT respectively.

The present study describes the normal cellular architecture and cell surface ultrastructure in different parts of the body of a hydra. The four areas studied – tentacles, hypostome, body column and stalk – show significant differences in the organization of epithelial cells and in the cell surface features. These distinguishing characters of each part of the hydra body correspond with the functional demands made on them. The tentacles, which, for example, play a crucial role in catching the prey, are endowed with batteries of nematocytes. Similarly, the hypostome, which ingests the prey, is supplied with specialized lip cells with sensory hairs.

In the present study, DMSO was used as a solvent for CB and taxol. DMSO at certain concentrations has been reported to induce changes in the hydra cell surface[15]. It was therefore necessary to confirm that the solvent controls treated with only DMSO were normal. In the present study, DMSO did not show any detectable effect on hydra ultrastructure at the dose used (8.5 µl/ml). This was the maximum dose of DMSO to which the CB- and taxol-treated hydra were exposed. In our earlier studies too, we have found DMSO not to have any significant effect on hydra[17].

Both CB and taxol were found to alter the cellular organization and cell surface ultrastructure all over the hydra body. In general, the effects of taxol were more drastic than those of CB. Cells in the tentacle and in the hypostome appeared more susceptible to the effects of the cytoskeletal drugs. This is not surprising since tentacles and hypostome need to have more contractile ability to fulfil their respective functions and are likely endowed with more MF and MT. The other body parts are also susceptible to CB and taxol as they too exhibit a great capacity to contract and expand albeit less than tentacles and hypostome. The present results show that alterations in the cytoskeletal components MF and MT lead to dramatic modulation of the cell surface. The results clearly demonstrate that the dynamic relationship between the cell surface and the cytoskeleton was established very early in evolution as a prelude to the complex processes of morphogenesis observed in the vertebrate embryo. In view of the discovery of homologs of a variety of vertebrate development-specific genes in hydra[18–21] (reviewed in ref. 4), the present results indicate that almost all the fundamental cellular and molecular processes required for the development of form and shape (morphogenesis) were in place in the diploblastic hydra.

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Presence of illites in Bay of Bengal – An analysis of the sample obtained from GEODROME

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GEODROME stands for Geophysical Deep-water Research Observatory for Multidisciplinary Experiment. The instrument can withstand up to a depth of 6000 m with its full operational conditions. GEODROME is a benthic station measurement consisting of several modular structures for research of geophysical and geochemical processes at the sea bottom. The multiparameter geophysical and geochemical measurements from the instrument would provide knowledge about earthquake processes taking place in the marine environment. It may also provide insight about the ongoing physical and chemical processes in the subsurface. The stand-alone recording has facilities to record data for long periods. In this study we have analysed the sample collected during our GEODROME experiment and some results are presented.

Keywords: Bay of Bengal, GEODROME, illite.

THE Indian continental margin is an important element in the evolutionary history of the break-up of the Indian Plate from Gondwanaland. The break-up between the east coast of India and Antarctica led to the development of the eastern continental margin of India followed by its subsidence and sediment deposition in the basins. The Bay of Bengal region is covered with pre- and post-collision sediments[1]. The nature of the crust beneath the Bay of Bengal is still controversial and it has been argued that either it is continental or oceanic type[2]. Controlled source seismic investigations delineated the sediment thickness at 13°N lat., which is of the order of 6–7 km and decreases to 2–3 km at distant fans[3]. Two prominent linear, geological features, viz. 85°E and 90°East Ridge have been identified in the Bay of Bengal. The 85°E Ridge is buried under sediments and geophysical investigations have traced its continuity 17°N, but the extension in the northern region is being debated.

A unique experiment to understand the nature of the crust and mantle in the Bay of Bengal area and to understand the genesis of the 85°E Ridge was taken up under Indo-Russian collaboration. The experiment envisaged the deployment of the Geophysical Deepwater Research Observatory for Multidisciplinary Experiment (GEODROME) in the Bay of Bengal during October 2003. The deployment was done using the Department of Ocean Development (DOD) Vessel *ORV Sagar Kanya*. Apart from understanding the genesis of the ridge, the equipment was itself being tested for sustenance in deep waters and in this aspect it was first type of experiment. Due to logistic constraints the instrument was deployed for short periods for the test run and retrieved. It was expected that receiver function analysis of the collected seismological data would provide one-dimensional configuration of the crust and mantle. Three broadband seismometers simultaneously operated on the eastern coast to calibrate the GEODROME. With the data from these equipment it was expected to resolve the nature and probably the physical characteristics of the underlying crust and upper mantle and thereby lay constraints on the geodynamics of this region.

The GEODROME consists of multicomponent geophysical as well as geochemical measurement units such as block for registration of seismic sensor, hydro-acoustic signals, magnetometer, salinity and temperature measurement units, broadband seismometer and hydrophone. These units are properly connected with connection cables and these cables are connected with a continuous DC power supply. The power supply with data recording units is placed within a spherical pressure chamber. The power supply is provided by a number of 1.5 V cells connected in series. These units are situated on a frame so as to sit on the ocean bottom as a single unit.

An Indian delegation visited Moscow during November 2000 to formulate the collaborative programme in certain specified branches of earth sciences. During the deliberation, a programme was signed for the development of GEODROME in the deep waters of Indian continental margins. The experiment was aimed at testing the operation of the GEODROME at water depths of around 3000 m. The project proposal included running of three observatories on the coast along with the deployment of the GEODROME in the Bay of Bengal.

A subsurface basement rise-like feature approximately parallel to 85°E longitude has been identified on the long-

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