

Webinar

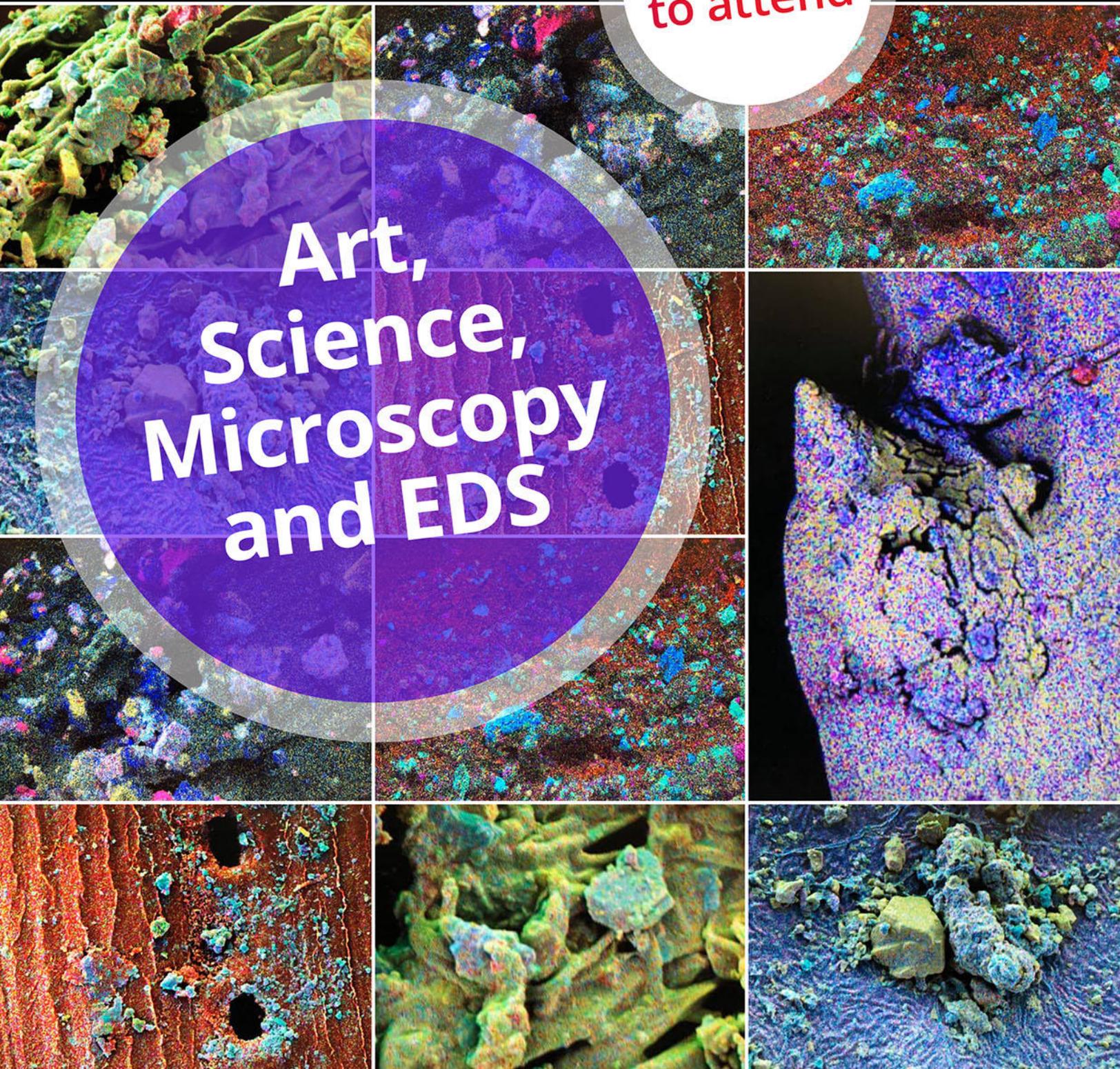
March 17, 2020

WILEY

OXFORD
INSTRUMENTS

Register
to attend

Art,
Science,
Microscopy
and EDS



Introduction to Studies on Cell Adhesion Using Invertebrate Models

SALLY P. LEYS

Department of Zoology, University of Queensland, Brisbane, Australia

Although much of today's knowledge of cell adhesion molecules comes from vertebrate (mammalian, avian, and amphibian) model systems, nonvertebrate models, specifically the slime mold, *Dictyostelium discoideum* (Bozzaro and Ponte, 1995), echinoderm (McClay et al., 1995; Gherzi et al. 1996), nematode, *Caenorhabditis elegans* (Gettner et al., 1995; Moulder et al., 1996), and fruit fly, *Drosophila* (Hynes, 1992; Roote and Zusman, 1996) have provided important insights into aspects of cell adhesion in development and morphogenesis, and have aided considerably with the characterization of the numerous molecules involved into the superfamilies of cadherins, selectins, integrins, and immunoglobulins (Pigott and Power, 1993; Steinberg, 1996). The advantages of these invertebrate model systems are their rapid rates of embryonic development, their amenability to genetic manipulation, the relative ease of developing mutants, and, quite importantly, the ease with which these animals can be maintained in the laboratory.

Comparative studies with invertebrates provide another avenue to research on cell adhesion. Considering that invertebrates make up some 95% of animal species, it is not surprising that a comparative approach has revealed an enormous diversity of molecules and structures involved in cell adhesion and has enhanced our understanding of the evolution of the molecules and mechanisms used. Since the first experiments that demonstrated species-specific cell sorting of dissociated sponge cells (Wilson, 1907), many invertebrate models have been used to investigate allorecognition and the immune response (e.g., tunicates: Parrinello et al., 1996; Raftos, 1996; Rinkevich, 1993; Sawada and Ohtake, 1994; annelids: Cooper et al., 1992; sponges: Yin and Humphreys, 1996). Other research has taken advantage of the ease with which some of these animals' cells can be dissociated into culture without the use of damaging enzymes or other agents, to examine cell-cell and cell-substrate interactions and cytoskeletal changes in vitro (Khan et al., 1997; Leys, 1997; Prysiezniak and Spencer, 1989) and in attempts to culture invertebrate cells for pharmacological and cell biological studies (Kawamura and Fujiwara, 1995; Rinkevich et al., 1994; Toullec et al., 1996). And the remarkable regenerative properties and ability of many invertebrates to reproduce asexually have been exploited to study aspects of cell adhesion during morphogenesis and embryogenesis (Ziegler and Stidwill, 1992; Zang and Sarras, 1994).

Through all studies on cell adhesion that use invertebrates runs the undercurrent of the evolutionary implications. The great diversity of cell adhesion structures and their constituent molecules found in invertebrates may be regarded as a glimpse of the history of cell adhesion. The invention of cell adhesion mechanisms

was a prerequisite for the evolution of multicellular life, and sponges, as the first multicellular animals to diverge during metazoan evolution, remain in many ways our earliest witnesses of the development of the first adhesion mechanisms and extracellular matrices. Through research into the evolutionary origin of cell adhesion molecules, it is becoming increasingly apparent that the cell adhesion and signaling molecules thought to have evolved to allow sophisticated regulation of processes in higher animals, are well established even in the "lowest" multicellular organisms (Brower et al., 1997; Müller, 1997; Müller, 1998).

In this special issue, some of the less well-known invertebrate models of cell adhesion systems are reviewed. The goals of this comparative approach to cell adhesion are to elucidate if possible the evolutionary origins of cell adhesion molecules known in higher organisms, to discover alternate roles of adhesion molecules, to demonstrate the usefulness of models that are "inexpensive and socially non-controversial" (Cooper et al., this issue), and finally, to discover novel adhesive systems. To this end the papers in this issue cover aspects of cell adhesion in (1) the immune response, (2) regeneration and morphogenesis, and (3) cell migration and adhesion/de-adhesion. They use a powerful combination of in vitro model systems and molecular techniques to determine the properties and roles of cell adhesion molecules in invertebrates such as sponges, cnidarians, earthworms, ctenophores, and tunicates.

In the first section, four papers discuss the role of cell adhesion molecules in the immune response. Classic studies on cell adhesion and histocompatibility in sponges are reviewed by Fernandez-Busquets and Burger. They provide a comprehensive account of the sponge "aggregation factor," the proteoglycan complex that is responsible for species specific aggregation of sponge cells, and discuss its relationship to cell adhesion molecules known from higher metazoans. Their experiments reveal that the genes coding for the aggregation factor are highly polymorphic, suggesting that this cell adhesion molecule is the functional equivalent to immunoglobulins in vertebrates.

In the second paper, Müller, Koziol, Müller, and Wiens describe an impressive number of adhesion molecules now characterized in sponges, including the well-known integrins, fibronectins, and collagens, as well as the cell surface receptors receptor tyrosine kinase (RTK) and proteins with the scavenger receptor cysteine-rich (SRCR) and short consensus repeats (SCR). They give powerful evidence from combined field and laboratory experiments to demonstrate that, like the aggregation factor, RTK shows Ig-like polymorphism. They show that SRCR, which may be the long-hunted-

for aggregation receptor of sponges, interestingly has an RGD domain, and therefore may interact with cell surface integrins. And finally, they describe the presence of the prophenoloxidase activating system in cells that die as a result of allorecognition. The evidence from sequence comparisons of numerous metazoan-specific proteins involved in cell adhesion in the Porifera convincingly shows that sponges were already well established as metazoans at the time of their divergence from the rest of multicellular animals some 600 to 800 million years ago.

Using purified proteoglycans—glyconectins—covalently linked to fluorescent beads, Misevic elegantly shows in the third paper, that these carbohydrates alone can cause homeotypic association of live and preserved sponge cells. He augments this work with atomic force microscope measurements that demonstrate the sheer strength of proteoglycan-proteoglycan interactions.

Cytotoxicity, cell-killing of foreign targets by natural killer cells, is one consequence of cell adhesion that may have evolved along with the appearance of multicellularity, suggest Cooper, Cossarizza, Kauschke, and Franceschi, who present the earthworm model in the fourth paper of this issue. Although annelids lack immunoglobulins, these were the first lower invertebrates in which measurable immunological recognition and memory was demonstrated. Cooper and colleagues cite extensive work showing that the earthworm coelomocyte or leukocyte expresses various cell differentiation (CD) antigens and functions as a natural cytotoxic cell, the equivalent of mammalian natural killer cells. They also give evidence for the presence of perforin, fetidins, lysenin, and hemolysin, lytic factors involved in cytotoxicity during the immune response in earthworms and other invertebrates. The growing repertoire of the molecules and events involved in the earthworm immune response, and the relative ease with which in vitro assays can be carried out with earthworm coelomocytes makes these invertebrates an excellent model system for immunological research.

The second theme of this issue looks at cell-cell and cell-substrate interactions during regeneration and morphogenesis. Schmid, Ono, and Reber-Müller use molecular and antibody techniques to study the role the mesoglea and extracellular matrix (ECM) ligands play in cell adhesion and cell migration during regeneration and morphogenesis in Hydrozoa. ECM proteins in the mesoglea are reviewed, and their roles in buoyancy, support, and even defense are discussed. Using in vivo and in vitro experiments, the authors show that the shape and the condition of the ECM is critical in determining the regeneration potential and the state of differentiation of isolated tissues.

As the highest known organisms capable of propagating by asexual reproduction, tunicates present a useful model for studying cell-cell adhesion during cell sorting and rearrangements such as occur during embryonic morphogenesis in a lower chordate. In the sixth paper of this special issue, Kawamura and Sugino provide an excellent overview of cell junctions and cell adhesion-related molecules in tunicates, emphasizing the role these molecules play in asexual reproduction.

Cytoskeletal responses to cell-substrate interactions are examined in vitro by Gaino and Magnino. Using primary cultures of cells gently dissociated from a calcareous sponge, they show that changes in adhesiveness at the cell perimeter largely determine directionality of cells during locomotion. Furthermore, not only are cells more likely to cross paths when in the presence of the cyclic nucleotide cAMP, but cells appear to find one another by following trails of torn fragments of cell membrane.

The last paper in the issue presents the delightfully novel adhesion system of lip closure in the ctenophore, *Beroë*. Tamm describes a transient reversible junction in beroid ctenophores that has parallels with the kind of reversible cell adhesion that takes place during developmental and wound healing processes in other animals. The opposed membranes in the ctenophore lip have a dense cytoplasmic coat of actin microfilaments that disassembles in milliseconds, probably the fastest known dynamic reorganization of actin known in the Metazoa. Such speedy rearrangement of the cytoskeleton, which then takes considerably longer to reassemble, is mediated by electrical signals triggered by chemo or mechanoreceptors on the lips.

The reviews in this issue are by no means intended to cover the all the issues in cell adhesion, but rather they provide thought-provoking up-to-date commentaries on contemporary topics within the broad field of cell adhesion that use invertebrate models. The emphasis on sponges as a model adhesion system is a reflection of their historic role in cell adhesion studies and their potential for use in future studies, and also of the editor's interests, biases, and limitations.

REFERENCES

- Bozzaro S, Ponte E. 1995. Cell adhesion in the life cycle of *Dictyostelium*. *Experientia* 51:1175–1188.
- Brower DL, Brower SM, Hayward DL, Bull EE. 1997. Molecular evolution of integrins: genes encoding integrin β subunits from a coral and a sponge. *Proc Natl Acad Sci USA* 94:9182–9187.
- Cooper EL, Rinkevich B, Uhlenbruck G, Valembos P. 1992. Invertebrate immunity: another viewpoint. *Scand J Immunol* 35:247–266.
- Gettner SN, Kenyon C, Reichardt LF. 1995. Characterization of bpat-3 heterodimers, a family of essential integrin receptors in *C. elegans*. *J Cell Biol* 129:1127–1141.
- Gherzi G, Salamone M, Levi G, Vittorelli M. 1996. Cell adhesion-dependent regulation of cell growth during sea urchin development. *Eur J Cell Biol* 69:259–266.
- Hynes RO. 1992. Integrins: versatility, modulation and signalling in cell adhesion. *Cell* 69:11–25.
- Kahn HR, Shivers RR, Saleuddin ASM. 1997. Cell junctions in the endocrine dorsal body cells of *Helisoma duryi* (*Mollusca: Pulmonata*): an in vitro study. *Tissue Cell* 29:39–46.
- Kawamura K, Fujiwara S. 1995. Establishment of cell lines from multipotent epithelial sheet in the budding tunicate *Polyandrocarpa misakiensis*. *Cell Struct Function* 20:97–106.
- Lays SP. 1997. Sponge cell culture: a comparative evaluation of adhesion to a native tissue extract and other culture substrates. *Tissue Cell* 29:77–87.
- McClay DR, Miller JR, Logan CY, Hertzler PL, Bachman ES, Matese JC, Sherwood DR, Armstrong NA. 1995. Cell adhesion and cell signaling at gastrulation in the sea urchin. *Theriogenology* 44:1145–1165.
- Moulder GL, Huang MM, Waterston RH, Barstead RI. 1996. Talin requires β integrin, but not vinculin, for its assembly into focal adhesion-like structures in the nematode *Caenorhabditis elegans*. *Mol Biol Cell* 7:1181–1193.
- Müller WEG. 1997. Origin of metazoan adhesion molecules and adhesion receptors as deduced from cDNA analyses in the marine sponge *Geodia cydonium*: a review. *Cell Tissue Res* 289:383–395.

- Müller WEG. 1998. Origin of metazoa: sponges as living fossils. *Naturwissenschaften* 85:11–25.
- Parinello N, Cammarata M, Arizza V. 1996. Univacular refractile hemocytes from the tunicate *Ciona intestinalis* are cytotoxic for mammalian erythrocytes in vitro. *Biol Bull* 190:418–425.
- Pigott R, Power C. 1993. The adhesion molecule factsbook. New York: Academic Press. 190 pp.
- Przysięzniak J, Spencer AN. 1989. Primary culture of identified neurones from a cnidarian. *J Exp Biol* 142:97–113.
- Raftos D. 1996. Interactions of the tunicate immunomodulatory proteins with mammalian cells. *Immunol Cell Biol* 74:26–31.
- Rinkevich B. 1993. Immunological resorption in *Botryllus schlosseri* (Tunicata) chimeras is characterized by multilevel hierarchical organization of histocompatibility alleles. A speculative endeavour. *Biol Bull* 184:342–345.
- Rinkevich B, Frank U, Rabinowitz C. 1994. Establishment of continuous cultures from cnidarians. *Marine Biol* 120:491–499.
- Roote C, Zusman S. 1995. Functions for PS integrins in tissue adhesion, migration and shape changes during early embryonic development in *Drosophila*. *Development* 122:1985–1994.
- Sawada T, Ohtake SI. 1994. Mixed-incubation of allogeneic hemocytes in the tunicate *Halocynthia roretzi*. *Zool Sci* 11:817–820.
- Steinberg MS. 1996. Adhesion in development: an historical overview. *Dev Biol* 180:377–388.
- Toullec JY, Crozat Y, Patrois J, Porcheron P. 1996. Development of primary cell cultures from the penaeid shrimps *Peneus vannamei* and *P. indicus*. *J Crustacean Biol* 16:643–649.
- Wilson HV. 1907. On some phenomena of coalescence and regeneration in sponges. *J Exp Zool* 5:245.
- Yin CQ, Humphreys T. 1996. Acute cytotoxic allogeneic histocompatibility reactions involving grey cells in the marine sponge, *Callyspongia diffusa*. *Biol Bull* 191:159–167.
- Ziegler U, Stidwell RP. 1992. The attachment of nematocytes from the primitive invertebrate *Hydra* to fibronectin is specific and RGD-dependent. *Exp Cell Res* 202:281–286.
- Zang X, Sarras MP Jr. 1994. Cell-extracellular matrix interactions under in vivo conditions during interstitial cell migration in *Hydra vulgaris*. *Development* 120:425–437.