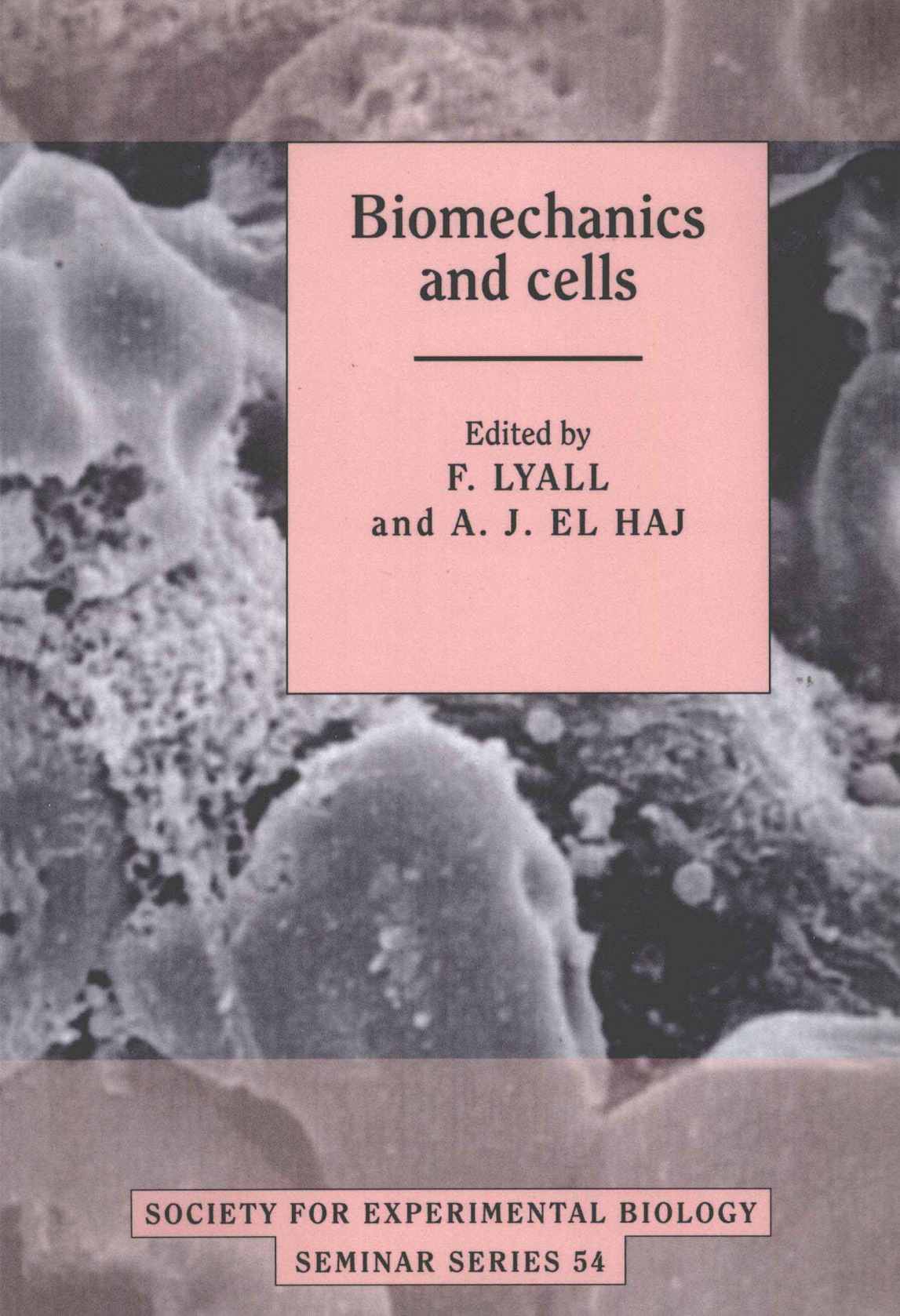

Biomechanics and cells

Edited by
F. LYALL
and A. J. EL HAJ

SOCIETY FOR EXPERIMENTAL BIOLOGY
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BIOMECHANICS AND CELLS

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Although the effects of exercise and mechanical forces on musculoskeletal and cardiovascular systems have been well documented, the actual mechanisms by which mechanical forces act at the cellular level are not well understood. At present, studies of the interaction of mechanical forces with cells encompass many different cell types in various tissues. This volume draws together these seemingly disparate observations and makes comparisons between the nature of the cellular responses in different tissues. Studies of cells derived from skeletal muscle, bone and cardiovascular tissue are considered, providing a comprehensive synthesis and review of recent work. The volume will be of interest to all those working in musculoskeletal and cardiovascular biology, as well as those taking courses in exercise and sports science, biomechanics and orthopaedics.

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BIOMECHANICS AND CELLS

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Part 1
Soft tissue

B. E. SUMPIO, W. DU, C. R. COHEN, L. EVANS, C. ISALES, O. R. ROSALES and I. MILLS

Signal transduction pathways in vascular cells exposed to cyclic strain

The importance of external physical forces in influencing the biology of cells is just being realised. Recent reports demonstrate that exposure of endothelial cells (EC) to a flowing culture media or to repetitive elongation can result in changes in morphology, proliferation and secretion of macromolecules (Dewey *et al.*, 1981; Davies *et al.*, 1984; Frangos, Eskin & McIntire, 1985; Sumpio *et al.*, 1987; Diamond, Eskin & McIntire, 1989; Sumpio & Widmann, 1990; Iba & Sumpio, 1991, 1992). Now, the major impetus in the field is to define the 'mechanosensor(s)' on the cells that are sensitive to the different external forces, the coupling intracellular pathways and the subsequent nuclear events which precede the cell response.

Mechanosensors

Cell surface sensors

The cell's plasma membrane, besides serving as a barrier to protect the cell interior, is the site of action and translation of external to internal signals. Although no 'strain-receptor' as such has been identified, it is clear that endothelial cells can 'sense' changes in pressure and strain. Furthermore, it is likely that this 'sensor' is located on the cell surface. The endothelial cell surface consists of multiple projections covered by a thin layer of glycocalyx (consisting mainly of glycoproteins, proteoglycans and derived substances). In an effort to characterise possible cell surface sensors, Suarez & Rubio (1991) perfused isolated guinea pig hearts with concavalin A or heparinase (agents which modify endothelial cell surface glycoproteins) and attenuated both the flow and pressure stretch induced rise in glycolytic flux normally seen in guinea pig hearts, while having no effect on basal glycolytic values. In contrast,

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infusion with hyaluronidase and chondroitinase (agents which modify surface proteoglycans) had no effect on flow-activated glycolysis, while modifying basal glycolytic values. Their data suggest that cell surface glycoproteins might play a role in 'sensing' changes in blood vessel flow and pressure.

Ion channels

Ion channels maintain the electrochemical balance, pH and osmolarity of the interior milieu of the cell. These channels span the cell membrane and are therefore subject to the mechanical stresses which affect the membrane. It would seem reasonable to presume that any force which affects the membrane tension may affect the channels within.

One model of mechanotransduction involving ion channels pictures the channel as a 'cylindrical plug' of protein embedded in the membrane (Kirber, Walsh & Singer, 1988) and predicts that in order for energy to activate the channel, it needs to be transmitted by cytoskeletal strings. Experiments utilising cytochalasins to disrupt the cytoskeleton have demonstrated an increase in channel sensitivity. The cytochalasins are postulated to act by cleaving *either* non-channel attachment sites in the membrane, thereby increasing the lattice spacing, or the parallel elastic elements in the membrane. The cell shape of capillary endothelial cells has also been shown to be determined by a counterbalance between the contractile forces of the microfilaments and the compression resistance of the microtubules (Ingber & Folkman, 1989). It suggests that membrane tension, and hence the state of the mechanosensitive ion channels, may be a function of the cytoskeletal elements.

Recent studies have also suggested the presence of mechanosensitive ion channels in vascular cells (for review see Davies, 1989). Endothelial cells have been shown to have 'stretch-activated' calcium-permeable channels using the cell attached patch technique. Using porcine aortic endothelial cells Lansman, Hallam & Rink (1987) found that by applying suction (10–20 mm Hg or 1.3–2.7 dynes cm^{-2}) to a cell attached membrane patch, a cation selective current (with a slope conductance of 19.1 pS and reversal potential of 17 mV when the patch electrode contained 110 mM $CaCl_2$) was observed which they speculated could carry sufficient calcium to serve as the second messenger in prostacyclin and EDRF (endothelium-derived relaxing factor) release. Stretch-activated ion channels have been described in other cell types (Guharay & Sachs, 1984; Morris, 1990; Davis *et al.*, 1992), including rat ventricular myocytes (Craelius, Chen & El-Sherif, 1988) and arterial baroreceptors (Schreiber *et al.*, 1971; Singh, 1982). The demonstration of a cation-selective channel

that is permeable to Ca^{2+} is not a universal finding since others have failed to confirm these channels in cultured endothelial cells from either bovine pulmonary artery, human umbilical vein or rabbit aorta (Adams *et al.*, 1989). In addition, Morris & Horn (1991) have recently suggested that, at least in the snail neurone growth cones, the single channel recordings of a potassium-selective stretch-activated channel may be an irrelevant artefact. Since they were unable to correlate their single-channel recordings with any macroscopic currents, it is as yet unclear whether these findings will pertain exclusively to snail neurones. In contrast, Davis *et al.* (1992) have reported that they were able to observe activation of a cation channel ($K^+ > Na^+ > Ba^{2+} > Ca^{2+}$; with a slope conductance of 7 pS when there was 110 mM $CaCl_2$ in the patch electrode) in both whole cell and single-channel recordings when 10–15% stretch (above control) was applied to porcine coronary artery smooth muscle cells.

Adragna (1991) studied the effect of cyclic stretch on sodium and potassium transport in bovine aortic EC (BAEC). He found an increase in Na^+ and K^+ content in cells stretched at 3 cycles min^{-1} and 24% strain for 7 days. The effect of ouabain and of ouabain plus furosemide suggested that cyclic stretch stimulated entry, or inhibited exit of Na^+ and K^+, or both. Further experiments using bradykinin suggested that cyclic stretch and bradykinin act on endothelial cells via an increase in intracellular calcium through release from intracellular pools and calcium entry into the cell. The intracellular calcium then behaves as a second messenger activating various ion channels to produce the observed ion shifts.

Further studies are required to investigate the importance and effect of mechanosensitive ion channels in vascular cells, which are subject to a continuously changing mechanical environment.

Intracellular coupling pathways

Once the endothelial cell has 'sensed' a change in the applied strain, the signal is 'transduced' to the cell interior. Multiple signal transduction pathways exist in vascular endothelial cells. This is not unexpected since the endothelium is metabolically active and many different inputs are processed simultaneously. However, it would be unlikely that one could activate a single second messenger pathway in an isolated manner, without this having important effects on the other pathways. For example, a rise in intracellular calcium can activate the calcium/calmodulin-dependent enzyme constitutive nitric oxide synthase, which in turn will stimulate the soluble guanylate cyclase and increase cGMP levels; this in turn may