that either pretreatment with dexamethasone had activated the DHEA receptor as well, or the configuration of the induced steroid receptor presented by *Tetrahymena* was equally suitable for binding fluorinated glucocorticoids and the less hormone-like DHEA, but not suitable for specific binding of testosterone.

The induced receptor also failed to bind ouabain and digoxin specifically. It rather showed a greater non-specific binding (e.g., increased binding in presence of cold hormone) similar to that observed on treatment of the control cells with triamcinolone. Digoxin and ouabain are non-hormones, but both have a sterane structure, which accounts for cross-reactions with dissimilar steroid hormones and non-hormones of sterane structure on the steroid receptors of newborn rats[14,15]. No such cross-reactions were detected in *Tetrahymena*.

Thus the present experimental observations permit the conclusion that the induced steroid receptor of *Tetrahymena* is not a general steroid receptor since, although it does specifically bind certain hormones, this specificity cannot be reconciled with either the functional or the structural categories of vertebrate hormones. The speculation that *Tetrahymena* would present induced receptors only to those steroid hormones which do naturally occur in its body[10,11] is not plausible either; it does in fact contain DHEA and, in a lesser amount, testosterone as well, but while it does bind DHEA, it fails to bind testosterone.

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Dystrophin and the integrity of the sarcolemma in Duchenne muscular dystrophy

C. J. Duncan [1]

Department of Zoology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX (England)
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Summary. It is suggested that in Duchenne muscular dystrophy the absence of dystrophin, which is probably a cytoskeletal protein underlying the sarcolemma, causes changes in stretch-activated cation channels rather than direct mechanical tearing of the surface membrane.
Key words. Dystrophin; calcium; skeletal muscle; muscular dystrophy.

The determination of the complete sequence of dystrophin, the missing protein product of human Duchenne muscular dystrophy (DMD)[2] shows that it is probably rod-shaped, about 150 nm in length and that it can be separated into four domains: (i) the N-terminal domain which is conserved with the actin-binding domain of α-actinin, (ii) a section that is predicted to be rod-shaped and formed of a succession of 25 triple helical segments similar to the repeat domains of spectrin and intermediate filaments, (iii) a cysteine-rich segment that is similar to the COOH domain of *Dictyostelium* α-actinin, (iv) a 420 amino acid C-terminal. Since dystrophin shares many features with spectrin and α-actinin, it has been suggested that it is a cytoskeletal protein[2] and it is also present in kidney, lung and brain, although its abundance is only about 1% of that in skeletal muscle[3]. Recent studies demonstrate that it is predominantly localised in the membrane of striated muscle cells[4–6], although earlier reports have indicated that it is mainly associated with the T-tubule system[7,8]. Dystrophin is also missing in the X-linked mouse muscular dystrophy, *mdx*[7]. These findings have led to suggestions that the function of dystrophin in normal muscle is to protect the membrane against the stresses associated with contraction[9] by providing mechanical strength for the sarcolemma[6]. Consequently, it is suggested that in DMD (where dystrophin is missing) the sarcolemma is subjected to local tearing[9], or locally separated from the basal lamina so causing focal lysis of the sarcolemma[6], or develops small gaps with the basal lamina preserved[4], or develops a membrane instability[5], causing focal plasmalemma breaks[10]. Such suggestions are consistent with the observed release of creatine kinase (CK) and other cytosolic proteins in DMD. These findings show interesting parallels with studies on eccentric contractions of human muscle where, after 20-min exercise, ultrastuctural damage was detectable and was more extensive in biopsy samples taken 24–48 h later. Contralateral muscles contracting concentrically were undamaged[11]. Muscles that are particularly affected in DMD are those that normally undergo eccentric contractions[11]. Cellular damage caused by eccentric contractions are also accompanied by large increases in plasma CK levels[12–14]; and it has been suggested that these damaging effects are also initially induced by mechanical damage to the sarcolemma[11,13,15].

A further similarity between these two types of muscle damage concerns selective fibre atrophy. Fast muscle fibres (Type II B) are preferentially affected in DMD[16] and only a small proportion of the fibres showed degeneration following eccentric contraction[13], although all fibres display equal staining for dystrophin[4–6].

Release of CK is also a typical feature of the experimental muscle damage in vitro that follows rises in intracellular calcium concentration where there is no mechanical damage to the sarcolemma; it is a characteristic of the oxygen- and calcium-paradoxes of the mammalian heart and of A23187-induced damage in skeletal muscle[17,18]. Cytosolic proteins

are found in the perfusate of the rat heart in the calcium-paradox within 90 s of the return of extracellular calcium. The molecular events at the sarcolemma associated with this release of cytosolic proteins is unknown, but there is, as yet, no firm evidence implicating rupture or tearing of the sarcolemma.

A significantly elevated intracellular muscle calcium concentration is present in DMD [15, 19–23] and these findings compare with the muscles of patients with malignant hyperthermia where myoplasmic free $[Ca^{2+}]$ is also raised significantly [24]. The sequence of events in malignant hyperthermia is known to be triggered by the release of Ca^{2+} from the sarcoplasmic reticulum when patients with this genetic syndrome are exposed to volatile anaesthetics [25] and a number of case reports show that confirmed malignant hyperthermia can develop during general anaesthesia of DMD patients [26–29]. There are clear parallels, therefore, between the aetiologies of DMD and malignant hyperthermia. Does elevated $[Ca^{2+}]_i$ precede and initiate the sarcolemma changes in DMD that are associated with the release of CK, or is it a consequence of them? Since myoplasmic $[Ca]_i$ is also markedly elevated in foetuses at risk of DMD, it is suggested that this is an early biochemical change and is the primary defect [20, 30, 31] i.e., as in experimental studies of muscle damage [18], $[Ca^{2+}]_i$ is elevated before the changes in the integrity of the sarcolemma and the consequent release of CK.

Dystrophin is also present in small quantities in brain, kidney and lung [3]. Prior to the isolation of dystrophin, the membrane properties of DMD erythrocytes had been intensively studied and, in spite of many conflicting reports [32], it is evident that structural, biochemical and physical differences exist in the cells from DMD patients; collectively these studies point to an abnormality in the membrane that is related to the control of shape and permeability [33]. In particular, DMD erythrocytes showed increased osmotic fragility [34–39], increased $Ca^{2+} + Mg^{2+}$-ATPase activity [37, 40–44], and increased phosphorylation of spectrin and band 3 protein [45–47]. Increasing Ca^{2+} entry in control erythrocytes by treatment with A23187 duplicates many of the changes in membrane properties seen in DMD [48]. Such findings suggest that there may well be changes in the cytoskeleton of the erythrocytes of DMD patients as well as at the sarcolemma.

Stretch-activated cation channels, which are gated by the expansion or distortion of the cell membrane, are of widespread occurrence in animal cells including amphibian [49] and avian [50, 51] skeletal muscle. There are differences in the relative cation-selectivity in different cells; in chick skeletal muscle the channels are relatively non-selective ($P_K/P_{Na} \simeq 2$ to 4) [51] whereas endothelial stretch-activated channels are about six times more permeable to Ca^{2+} than to Na^+ [52] and the epithelium of the choroid plexus possesses a cation-selective, Ca^{2+}-permeable channel which opens with membrane stretch in hypotonic media, thereby permitting Ca entry [53]. The cation channel in frog lens epithelia is responsive to pressure and Ca^{2+} [54].

The function of these stretch-activated cation channels and their role in the evolution of ion channels remain unclear, but Kullberg [55] has suggested that ion channels involved in osmoregulation may have been among the first to evolve. The properties of such channels in three different cells illustrates their different roles in cellular physiology. The cation-selective, Ca^{2+}-permeable channel of the choroid plexus epithelium which opens with membrane stretch when swollen in hypotonic media is apparently involved in volume regulation by Ca^{2+}-influx [31]. When the tonicity of the bathing solution of Purkinje strands was reduced to 75% of normal, the cells depolarized by some 3 mV but $[Ca^{2+}]_i$ fell much more than that predicted by simple osmotic dilution. Increased tonicity to 150 or 200% caused a hyperpolarization of the resting potential but $[Ca^{2+}]_i$ showed a dramatic two- or four-fold increase respectively [56]. Non-confluent fibroblasts are relatively depolarized when compared with confluent fibroblasts and transiently hyperpolarize when subjected to mechanical, electrical or chemical stimulation. This electrical activity ceases when the cells become confluent. Stretch-activated channels have now been described in non-confluent cells which would serve for the transduction of mechanical stimuli; they are permeable to Na^+ and K^+ and are novel in exhibiting adaptation as well as sustained activity in response to suction. The high Na^+-permeability suggests that channel stimulation causes depolarization, and increases in intracellular Na^+ and Ca^{2+} are central to current theories describing the activity and membrane potential changes in non-confluent fibroblasts. The location of these stretch-activated channels at the cell border, where continued movements occur, suggests that they are probably involved in stress measurement during cell movement rather than volume regulation [57]. Membrane-permeability of fibroblasts is known to be modified in DMD (see below).

The cation channels in chick skeletal muscle become markedly more sensitive following treatment with cytochalasin B (which binds with the F-actin of cytoskeletal microfilaments), with the stretch-sensitivity constant increasing from 0.08 to 2.4 $(\text{dyn cm}^{-1})^{-2}$ and it is suggested that the channels gather force from a large area of membrane via a cytoskeletal network [50]. It is noteworthy (see below) that the anion channel of the red cell plasma membrane (the transmembrane Band 3 protein) has its cytoplasmic domain anchored to the cytoskeletal network via ankyrin and recently it has been shown that the voltage-dependent sodium channels from rat brain associate with ankyrin and spectrin; it is suggested that ankyrin links these neuronal cation-channels to the underlying cytoskeleton [58].

It is therefore suggested that DMD muscle lacking dystrophin is analogous with normal muscle treated with cytochalasin B or with muscle subjected to excessive eccentric contractile activity. Contraction activates stretch-sensitive ion-channels, which are not necessarily Ca^{2+}-permeable; increases in Na^+-permeability would produce depolarization and promote excitation-contraction coupling and Ca^{2+}-influx. Skeletal muscles of dystrophic mice have a reduced $[K^+]_i$ and a marked fall in resting potential [59, 60], with mean values of -59 mV compared with control values of -70 mV [61]. Membrane specific conductance was greatly increased in murine dystrophic muscle, mainly because of a specific increase in Cl^- conductance [62], which compares with an increased Cl^- permeability in DMD fibroblasts [63] and an increase in input conductance in DMD intercostal muscle [64]. Thus, lack of a membrane cytoskeletal protein in DMD may also cause changes in anion-permeability. It is also clear that dystrophic muscle exhibits changes in the electric properties of its membrane which precede physical damage (tearing, release of creatine kinase) to the sarcolemma. Thus, depolarization and conductance changes may lead directly or indirectly to increases in Ca^{2+}-influx which, in turn, activate the independent pathways that lead to sarcolemma breakdown (and release of CK) and to myofilament damage [18]. If the absence of dystrophin in DMD produces changes in cation-permeability of the muscle, it may be possible to ameliorate the condition therapeutically [17]; if absence of dystrophin directly causes tearing of the sarcolemma, a different strategy will be required, including changes in the programme of physiotherapy as suggested by Edwards et al. [15]. In any event, it would probably be beneficial to explore ways in which the adverse effects of eccentric (and concentric) contractions in DMD could be ameliorated, as proposed by these workers.

Note added in proof. Stretch-activated ion channels, which conduct Ca^{2+} as well as K^+ and Na^+, have been described

in toad smooth muscle[65] and $[Ca^{2+}]_i$ is elevated in skeletal muscle of *mdx* mice which results in enhanced net protein degradation[66].

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