The rapid advances in knowledge of the dystrophin gene and its function make it important to appraise from time-to-time the developments in the basic sciences for their clinical implications. To this end the Italian National Academy (Accademia Nazionale dei Lincei) and the British Council sponsored, with help from the Italian Muscular Dystrophy Association, a meeting on 20 and 21 February (1992) in Rome at the Palazzo Corsini. Although principally an Anglo-Italian occasion the programme was strengthened by invited papers from leading workers in the field from other countries.

Genes and their products

The introductory talks by D. J. Blake presenting the work of the Oxford group and D. Yaffe from the group at the Weizman Institute (Rehovot, Israel) concentrated on the genes concerned in the synthesis of dystrophin and dystrophin related proteins. From the two genes coding for these proteins seven different gene products have so far been identified. The dystrophin gene located on the X chromosome encodes about 74 exons and gives rise to at least five different proteins. The muscle isoform is the predominant form with lower amounts in cardiac muscle. In brain neurones are smaller amounts of an isoform that has a different first exon from that of the muscle protein and is produced by transcription using an alternative promoter in a tissue specific manner. There is also evidence of another isoform associated with the Purkinje cells of the cerebellum. In addition to these proteins of approximately 400 kDa the normal dystrophin gene also gives rise to smaller proteins. Rat Schwannoma and human glioma cells do not produce full length mRNA in detectable amounts but express a 4.8 kb mRNA that is transcribed from a cDNA derived from the 3′ end of the dystrophin gene but with a unique 5′ untranslated region absent from the 14 kb transcript. The Oxford group have named the gene product from the 4.8 kb mRNA transcript, apo-dystrophin-1, and that from a 6.5 kb transcript identified only in a rat C6 glioma cell line, apo-dystrophin-2. The transcript for the apo-dystrophin-1 was first discovered in liver by the Weizman group who consider it to be the major product of the dystrophin gene in non-muscle tissues. It is a protein of 71 kDa consisting of the two C-terminal domains of dystrophin and is localized in the membrane. The Weizman group have now studied the cDNA corresponding to the 71 kDa protein in some detail to determine the mechanism of its formation from the dystrophin gene and have developed translation vectors to produce the protein in the quantities required for its study. Although the 71 kDa protein and apo-dystrophin-1 are apparently identical there is a discrepancy in the size of the mRNA reported by the two groups.

Progress on the study of the 13 kb mRNA encoded on chromosome 6 was reported by the Oxford group. They suggest that the gene product originally known as dystrophin related protein should be named 'utrophin'. As an interim measure there are some advantages in giving the members of this group of proteins unique individual names. Nevertheless it might be wiser to leave the final decision on nomenclature until such time as we have more details of the complexity of the dystrophin group of proteins, the interrelations between the members and their functions. Most of the cDNA of utrophin has been cloned and the evidence available indicates that the gene probably spans about 2000 kb of chromosome 6 with the genomic organization similar to that of dystrophin. The C-terminal domain has 70% homology with dystrophin but there is only 20–30% in the spectrin domain. It is expressed in larger amounts of fetal tissue and has been located at the myoneural junction. In normal muscle utrophin is not present in the sarcolemma but appears to be up-regulated in Duchenne muscular dystrophy.

The fact that all seven transcripts of the X chromosome and chromosome 6 located genes so far identified have considerable homology in the C-terminal domains, and possibly elsewhere, is an important consideration in selecting probes for clinical diagnosis. Nucleic acid and antibody probes must be absolutely specific for the regions of the gene, its transcript and product, that are unique to the isoform which is under investigation. It seems likely that this was not always the case in the past.

Structure and function of dystrophin

The session on the cytoskeleton and the functional role of dystrophin was introduced by a general review from P. C. Marchisio of the interactions between the extracellular matrix and the cytoskeleton. At this stage it is not known whether dystrophin is associated with the adhesion plaques, podosomes, to which particular attention was paid in this talk. The evidence currently available indicates that the dystrophin molecule exists in a form that is about 120–30 nm long. In the course of summarizing the Japanese contributions to research on Xp21 myopathies S. Ebashi described electron microscope studies on the isolated protein from K. Maruyama’s group
(Chiba, Japan) that suggested it could exist as an antiparallel tetramer of that length. A similar value for the molecular length in situ was reported by M. Cullen (from the Newcastle group) using gold conjugated antibodies to specified regions of the dystrophin molecule. By using antibodies directly labelled with gold it has been confirmed that the spectrin domain of dystrophin lies 15 nm from the membrane to which the C-terminal is attached. The Newcastle group also reported occasional staining of other membranes and fine filaments within the cell but the significance of these findings is not clear. Dystrophin could not be detected in the intercalated discs of cardiac muscle nor in the attached plaques of smooth muscle.

The pattern of association of dystrophin with the proteins of the cytoskeletal-membrane is slowly beginning to merge. S. V. Perry described proton NMR studies that have enabled two N-terminal sites of dystrophin involved in interaction with actin to be identified. The corresponding binding sites on actin have also been characterized and are probably the same as those that can bind α-actinin and β-spectrin. Unlike the latter protein the spectrin domain of dystrophin does not contain an actin-binding site suggesting that this property is confined to the N-terminal region of dystrophin. Evidence for the binding by dystrophin of actin was also presented by G. Salvati who reported that talin, but not calcium, could bind to this protein. Membrane-bound dystrophin is a substrate for a variety of protein kinases but not for endogenous tyrosine kinase.

The dystrophin gene is remarkable in the range and location of the deletions it exhibits in the Xp21 myopathies. Detailed studies of the deletions using cDNA probes and antibodies with specificities for defined regions of dystrophin can provide information about the properties of the gene and hint at those regions of the molecule of particular functional significance. A study reported by G. A. Danielli on data collected by diagnostic centres in Europe indicates that there were approximately three times as many deletions occurring distal to exon 40 than in the region proximal to this gene. There were suggestions from the data that preferential breakpoints might lie in introns 43, 44, 49, 50 and 53, with a bias to a particular breakpoint being apparent in different populations. Louise Nicholson directed attention to the observation that dystrophin can often be detected in Duchenne patients whose dystrophin genes exhibit frame shift deletions. These would be expected to lead to the synthesis of severely truncated proteins that are rapidly broken down in the muscle. Nevertheless 60% of the Duchenne patients exhibited clear dystrophin-positive fibres, although such fibres were usually less than 1% of the total. A phenomenon referred to as ‘reversion’. Using a monoclonal antibody to the spectrin domain the dystrophin content of biopsy muscle samples from patients with Xp21 myopathies was estimated to range 7–73% of the normal. These values were in good correlation with the severity of the clinical conditions. It was suggested that dystrophin synthesis could occur in Duchenne patients by ‘exon skipping’. If indeed the Duchenne muscle cell has this capacity to make a slightly shortened dystrophin molecule with alleviation of the clinical condition the possibility of its exploitation should be examined.

Approach to therapy

The current interest in myoblast transfer as a therapy in the Xp21 myopathies has focussed attention on muscle precursor cells in general. An additional stimulus to research into muscle regeneration and development is the remarkable fact that the mdx mouse after a period of impaired muscle function in early life is able to overcome the absence of dystrophin and function almost normally. This is widely considered to be due to the highly developed capacity of mouse muscle to regenerate. As myogenic cells isolated from embryonic, fetal and adult tissues express different phenotypes in vitro it is clearly important to decide which type of cell is most suitable for myoblast transfer. The possibility that the differential expression of the recently discovered myogenic regulatory genes might be responsible for the phenotypic differences observed between myoblasts was examined by G. Cossu. He showed that embryonic, fetal and adult (satellite) myoblasts expressed the myo D1 and myogenin gene products in similar amounts. In contrast primordial myoblasts in somites undergo terminal differentiation without expression of these gene products. No differential effect of growth factors on the proliferation of embryonic and fetal myoblasts could be demonstrated. In this context it is of interest that myotubes can be shown to release a factor in the medium that supports myoblast proliferation. In many ways the regenerative capacity of the mouse muscle is more comparable to that obtained with young animals. Similar effects of age on the regenerative capacity of muscle from Duchenne patients have been reported in the literature.

Although it has been clearly demonstrated that myoblast transfer can lead to production of muscle fibres containing dystrophin in a muscle from which this protein is normally absent, there are still many problems in using this procedure as an effective therapy. T. Patridge from the Charing Cross Medical School (London, UK) discussed investigations aimed to make the procedure more efficient. After injection of myoblasts into mdx mice the dystrophin-positive myotubes tend to be localized around the injection site and often there is a patchy distribution of dystrophin in the membrane of the new fibres. Indeed it might be argued that for the myotube to be restored to normal function it requires an even