IDENTIFICATION OF *TETRAHYMENA* 14-NM FILAMENT-ASSOCIATED PROTEIN AS ELONGATION FACTOR 1α

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Tetrahymena 14-nm filament-forming protein has dual functions as a citrate synthase in mitochondria, and as a cytoskeletal protein involved in oral morphogenesis and in pronuclear behavior during conjugation. The 14-nm filament protein fraction prepared by assembly and disassembly contained two 49-kDa proteins whose isoelectric points were 8.0 and 9.0. Monoclonal antibodies against the 14-nm filament protein fraction were clearly separated into two groups reacting to pI 8.0 or pI 9.0 protein specifically. From the N-terminal amino acid sequence, the pI 8.0 protein was identified as the 14-nm filament-forming protein. The pI 9.0 protein is considered to be a 14-nm filament-associated protein since the pI 9.0 protein copurifies with the pI 8.0 protein during two cycles of an assembly and disassembly purification protocol. Cloning and sequencing the pI 9.0 protein gene from a *T. pyriformis* cDNA library, we identified the pI 9.0 protein as elongation factor 1α (EF-1α) based on it sharing 73-76% sequence identity with EF-1α from several species.

THE BINDING SITE FOR COLLAGEN FIBRILS ON A CELLULAR FIBRONECTIN (cFN) MOLECULE

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The collagen gel culture is a useful experimental system in which the mode of binding between fibroblasts (Fbs) and collagen fibrils (CL) can be analyzed. With this culture Fbs recognize CL through cFN but not plasma FN (pFN). We have obtained monoclonal antibody (A3A5) which inhibits human Fbs-mediated collagen gel contraction and recognizes human FN. Quantitative assays of the extent of Fbs spreading on CL also suggested the involvement of cFN in binding of Fbs to CL. The region in FN recognized by A3A5 was analyzed. Human pFN was digested with trypsin and was subjected to immunoblot analyses with A3A5. The shortest fragment with molecular weight 19.5 kDa which reacts with A3A5 was analyzed for the to amino acid sequence and was found to have a sequence of TAGPDQT at the N-terminal region. From this we suppose that the binding site of CL on cFN is localized between typeIII region on the N-terminal side of ED-A and the middle of Heparin domein. For more precise analyses on the recognition site of A3A5 were carried out using human FN cDNAs encoding cFN and pFN. FN cDNAs fragments contained resion of 19.5 kDa trypsin fragment were constructed in vector and expressed as a fusion protein. This protein is now under investigation for the reactivity with A3A5.

PRIMARY STRUCTURE AND PROPOSED TERTIARY STRUCTURE MODEL OF THE SQUID SODIUM CHANNEL.

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The complete amino acid sequence of a sodium channel from squid *Loligo bleekeri* has been deduced by cloning and sequence analysis of the complementary DNA. The deduced sequence revealed an organization virtually identical to the vertebrate sodium channel proteins; four homologous domains containing all six membrane-spanning structures are repeated in tandem with connecting linkers of various sizes. A unique feature of the squid Na channel is the 1,522 residue sequence, approximately three fourths of those of the rat sodium channels I, II and III. On the basis of the result, we have proposed a tertiary structure model of the sodium channel where the transmembrane segments are octagonally aligned and the four linkers of S5-6 between segments S5 and S6 play a crucial role in the activation gate, voltage sensor and ion selective pore, which can slide, depending on membrane potentials, along inner walls consisting of segments S2 and S4 alternately. The proposed model is contrasted with that of Noda et al.. The proposed model could explain various unsolved phenomena, for example, effect of TTX (tetrodotoxin) on the gating current of the sodium channnel.

BREAKDOWN OF LARVAL TISSUES OF AMPHIBIAN TADPOLE BY ASPARTIC PROTEINASE.

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Amphibian tadpoles undergo metamorphosis and convert themselves into frogs by destroying their larval tissues and developing adult ones. The activity of acidic proteinases increases markedly in metamorphosing tail tissue. They are one of key enzymes in this histolysis.

This study examined the activity of aspartic proteinase in tissues of tail, limbs and dorsal body muscle of metamorphosing bullfrog tadpole (Rana catesbaiana) in relation to changes in DNA contents. The results suggested that the process of tissue degradation is divided into two phases. In the first phase activity of aspartic proteinase increased which was observed in both tail and dorsal body muscle at Taylor and Kollros stage XXI. This phase did not show any changes in the DNA contents per tissue weights. In the second phase which was found only in tail tissue (especially strong in distal part of tail) at T-K stage XXII, the activity further increased with a concomitant rise of DNA content which is most probable due to tissue condensation. The first phase may reflect autolysis, while the second is heterolysis probably caused by invading macrophages. The enzyme activity in limbs remained quite low during metamorphosis.