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The three dimensional structure of polycystin 1, gene expression from alternate coding frames in pkd1 and autoimmunity

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Polycystin 1 (Pc-1) is an exceptionally large multicomponent protein with numerous cytosolic components and an eleven-stranded integral membrane domain. We are determining the three dimensional structure of Pc-1 by combining crystallographic and computer modeling techniques. By comparing the amino acid sequences of sixteen structural domains in Pc-1 with structures in the protein data bank (PDB) we have been able to map PDB structures to 41% of the 4303 amino acids in Pc-1.

The lectin binding domain of Pc-1 was found to have sequence identity with three PDB structures, a sugar binding protein complexed with mannose (1SL4, 27% sequence identity), a toxin (1FVW, 30%) and a c-type lectin complex with galactose (1TLG, 14%). A superimposed model of the three PDB structures revealed the presence of a half dozen conserved residues critical to the protein fold including two disulfide bridges. It has been demonstrated that the c-type lectin domain of Pc-1 binds carbohydrates in a calcium dependent manner (Weston, B., et. al. Biochim. Biophys. Acta 1536. (2001):161-176). Using these data we have been able to model the three-dimensional structure of the c-type lectin including the residues that probably interact with the carbohydrate molecule and Ca+ ions found in the c-type lectin complex with galactose.

We have discovered that 20% of the genes in the SWISS PROT/TrEMBL database have multiple open reading frames and a statistically significant bias in nucleic acid triple composition (Duax, W., et al., Protein, Structure, Function and Bioinformatics, 61. (2005):900-906). In the course of attempting to clone and express domains of the Pc-1 gene, several fluorescently tagged peptides have been expressed from the antisense strand of the gene. Antibodies to peptide sequences from a frame shift mutant of the last 90 amino acids of the carboxyl terminal of Pc-1 have been shown to colocalize with fibrous deposits in PKD cysts (Van Adelsberg, J. and Frank, D., Nature Med, 4. (1995):359-364). Peptides expressed by the antisense strand of the human autoantigen protease-3 have been found to induce antibodies in patients with inflammatory vascular disease, (Pendergraft III, W., et. al. Nature Med 10. (2004):72-79) suggesting that peptides derived from alternate reading frames of a gene may play a critical role in autoimmune disease.

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Two different lipoamide dehydrogenases (E_3s) from *Thermus thermophilus*: Crystal structures and their evolutionary relationship

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2-Oxoglutarate dehydrogenase complex (OGDC) and pyruvate dehydrogenase complex (PDC) have similar architectures composed of three proteins, E_1, E_2 and E_3. It is known that E_1 and E_2 are substrate-specific while E3 is the common component of these complexes. Recent genome sequence analyses of several organisms, however, showed that those E_3s could be divided into two types, E_3o and E_3p, depending on the location in the operon. Furthermore, it has been found that a few of organisms possess two E_3 genes, and we have found that they correspond to E_3o and E_3p, suggesting that the two different E_3s are bound to the cognate complexes. To compare the two structures, the crystal structures of E_3o and E_3p from *Thermus thermophilus* have been determined at 1.7 and 1.6 Å resolutions, respectively. As expected from their sequence homology, the overall structures are similar to each other. The structures of the active sites are highly conserved between the two E_3s because both may catalyze the oxidation/reduction reactions of lipoyl groups in respective complexes. Several differences are found on surface residues, which form flexible loops that may be in contact with the different core architectures of the complexes. To clarify the core structures of the complexes, ultracentrifugation experiments of E_2o and E_2p were carried out. It revealed that the cores of OGDC and PDC in *Thermus thermophilus* are cubic (432 symmetry, $24E_1$:$24E_2$:$12E_3$ composition) and icosahedral (532 symmetry, $60E_1$:$60E_2$:$24E_3$ composition), respectively. These structural features are similar to those of eucaryotes and Gram-positive prokaryotes, but different from those of Gram-negative prokaryotes where both OGDC and PDC are cubic. We propose that an ancestor operon with a cubic symmetry carrying a set of E_1, E_2 and E_3 genes may have taken the following evolutionary steps: (1) duplication to generate two operons, (2a) disappearance of one of the E_3 genes in either operon (in Gram-negative prokaryotes), or (2b) transformation of the architecture in one of the operons into the icosahedral form (in *Thermus thermophilus*), and (3) disappearance of one of the E_3 genes in the operon with a cubic architecture (in Gram-positive prokaryotes and in eucaryotes).