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Towards Atomic Detail of Intermediate Filament Structure

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Intermediate filaments (IFs) are principal components of the cytoskeleton in higher eukaryotic cells. The way the elementary IF dimers consisting of a head, coiled-coiled rod and tail domains assemble into a filament is currently poorly understood. However, mutations in these proteins are known to be responsible for a number of currently uncurable human diseases such as myopathies, cardiopathies, neuronal and skin diseases. We are currently working on the 3D structure of several different types of IFs, as well as on the mechanism of the disease-related point mutations that distort the IF structure.
With desmin present in muscle cells, we are focussing on mutations in the 2B coil of its rod domain that are linked to myopathies. Most of these are mutations to proline expected to distort the α-helical structure. Multiple 2B coil fragments of about 60 amino acids were purified and screened towards obtaining crystals suitable for X-ray analysis. We are also trying to obtain the crystal structure of the desmin fragment carrying the E401S mutation which disrupts a predicted interhelical salt bridge.
The assembly of lamins representing the nuclear IF proteins starts with the longitudinal association of dimers with a short N-C overlap of their rod domains (rather than with a lateral association typical for cytoplasmic IFs). To investigate the assembly of lamins A, B1 and B2 at the molecular level, we have prepared 13 recombinant N- and C-terminal fragments of these proteins. These fragments assumed to represent the minimal regions of the longitudinal association were analysed using analytical ultracentrifugation and circular dichroism. We show that the most of the studied N-terminal fragments from lamins A and B1 form dimers. Furthermore, we demonstrate that the N- and C-terminal fragments mixed at 1:1 ratio are able to form heterotetramers, mimicking the N-C overlap. Remarkably, N-terminal constructs from lamin A may associate with C-terminal constructs from lamin B1 and vice versa. This is of considerable importance for the understanding of lamin assembly within the nuclear lamina. Moreover, the N-terminal fragments lacking the short head domain can still form stable tetramers with the C-terminal ones, suggesting that the head is not involved in the complex formation. Crystallisation trials with lamin fragments and their heterotetrameric complexes are in progress.

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Possible interactions between human mitochondrial thioredoxin 2 and human peroxiredoxin 5

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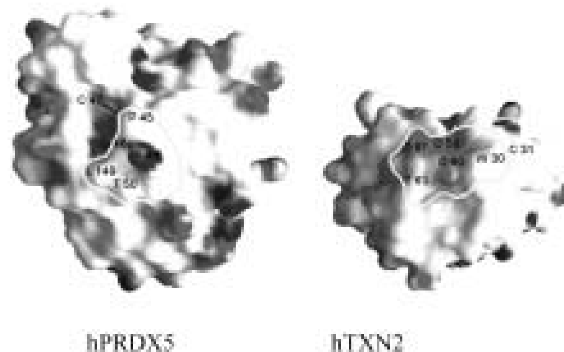
Thioredoxins (TXNs) are redox proteins characterized by a conserved active site sequence Cys-Gly-Pro-Cys. The catalytic cysteines of TXNs maintain thiols of protein substrates in a reduced state and thereby become oxidized themselves. In order to understand the recognition mechanism between human mitochondrial thioredoxin 2 (hTXN2) and one of its target protein in mitochondria, peroxiredoxin 5 (hPRDX5), profit was reaped from the availability of the crystal structure of *Haemophilus influenza* hybrid Prx5 [1]. This hybrid Prx5 structure reveals the presence of two domains, a Prx5 domain homologous to hPRDX5, and a glutaredoxin (Grx) domain. Like TXN, Grx is an electron donor protein, capable of reducing an oxidized target protein. Interestingly, in this crystal structure, Prx5 and Grx domains belonging to different monomers interact with each other and are both in the reduced form. This interaction exists independently on redox conditions. It can thus be postulated that it can exist before the establishment of the disulfide bond to be reduced in the target protein. For simulating a similar interaction between reduced hTXN2 and reduced hPRDX5, the reduced molecule of hTXN2 [2] was superposed on Grx and human PRDX5 [3] on Prx5 of the hybrid Prx structure. In both cases, this structural alignment resulted in a good spatial coincidence of the cysteine residues of the active sites. The contact surfaces involved in the formation of this complex are contoured by a yellow line on the figure. A negatively charged cavity at the surface of hTXN2 comes in contact with a positively charged protuberance of hPRDX5, which could fit the cavity of hTXN2. The remaining contacts are mainly hydrophobic.



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