The RecBCD enzyme binds at the broken DNA end and rapidly unwinds the duplex in an ATP-dependent manner. As unwinding proceeds, the 3' terminated strand is continuously degraded by an endonuclease activity. This unwinding and digestion continues until the Chi sequence is encountered, at which point the enzyme pauses, and the nuclease switches to the other DNA strand. The enzyme then continues unwinding the duplex, digesting the 5' terminated strand and loading the RecA strand-exchange protein onto the 3' strand, now capped by the Chi sequence (**Figure 76**) in preparation for the next stage of HR.

Considerable amounts of biochemical and genetic data have been obtained on the RecBCD system. Recent results have shown that the enzyme has a bipolar helicase activity, with one motor translocating along each strand, each running with opposite polarity [2]. It has been shown that the 3'-5' and 5'-3' helicase activities reside with the RecB and RecD subunits respectively, the nuclease is associated with the C-terminal domain of RecB and Chi recognition occurs in RecC.

We have recently determined the crystal structure of RecBCD bound to a blunt-ended DNA hairpin using data collected on ESRF beamlines **ID14-1** and **ID14-4**. The structure was solved in the absence of ATP and so represents an initiation complex prior to translocation (**Figure 77**). Despite this, the DNA substrate has been partially unwound, with four base-pairs of the duplex split.

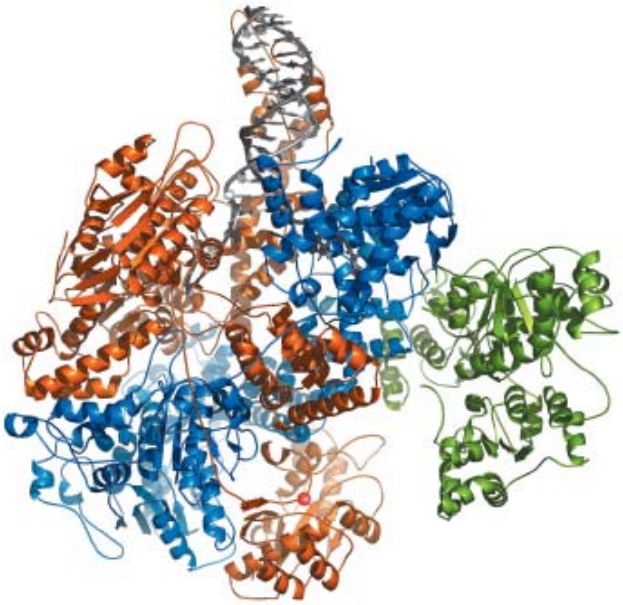

Fig. 77: Ribbon diagram of the RecBCD complex. RecB is shown in orange, RecC blue, and RecD green. The two strands of DNA (grey) are split at the top of the protein and enter tunnels directing the 3′ tail to the RecC Chi-recognition site and nuclease, and the 5′ tail to RecD. The active site of the nuclease is highlighted by a bound calcium ion, depicted as a red sphere.

As expected from sequence analyses, both RecB and RecD have folds characteristic of superfamily I DNA helicases, with the C-terminal nuclease domain of RecB attached by a long flexible linker. In the crystal structure a calcium ion replaces the native magnesium ion in the nuclease active site. The RecC protein forms a plate-like scaffold around which the RecB is tightly wrapped, while RecD maintains a looser association. RecC has three channels running through it; a large central cavity responsible for binding RecB, and two smaller channels for each of the DNA tails. These channels guide the 3' and 5' tails towards the nuclease and RecD respectively. Intriguingly, the 3' channel is partially formed from the N-terminal domain of RecC, which also has a characteristic helicase fold, a fact not apparent from the sequence. This observation suggests that RecC is in fact a defunct helicase, which has evolved the ability to recognise the Chi sequence. In the current crystal structure, the nuclease is ideally positioned to accept the 3' tail of the DNA following its passage through RecC. Presumably the Chi sequence induces some conformational switch, which alters the nuclease strand specificity as well as activating the RecA loading process.

These structural studies have shown how two helicases can combine to form a highly efficient molecular machine and provide a basis for an elaborate regulated nuclease system. Unravelling the details of this control mechanism should prove to be a rewarding exercise.

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Principal Publication and Authors

M.R. Singleton (a), M.S. Dillingham (b), M. Gaudier (a), S. C. Kowalczykowski (c), D.B. Wigley (a), *Nature* **432**, 187-193 (2004).
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The Structure from Microcrystals of the Cross-β Spine of Amyloid Fibrils

Decades of research by medical and biological scientists have established that neurodegenerative amyloid diseases, such as Alzheimer's, are each associated with a particular protein that converts into fibrillar form. Although these various proteins differ in sequence and

three-dimensional structure, their fibrils share common properties: they are ~ 80-150 Å in diameter, highly elongated and unbranched; they bind flat dyes such as Congo Red and display a characteristic birefringence, showing that they harbour an apolar, periodically arrayed environment; they give a characteristic "cross-β" diffraction pattern, reflecting extended protein (beta) strands, perpendicular to the fibril axis, spaced about 4.8 Å apart. These similarities point to a common structure for the spine of all amyloid fibrils, including the "amyloid-like" fibrils formed from globular proteins by removing them from their native conditions. This common structure resisted detailed elucidation in the past because of the difficulty of extracting atomic-level detail from fibrillar samples, either by NMR or X-ray diffraction.

Our road to the atomic structure of the cross-β spine had to surmount two major barriers. The first was crystals. We selected the yeast protein Sup35 for X-ray crystallography because extensive studies in other laboratories have shown that its formation of amyloid-like fibrils is the basis of protein-based inheritance and prion-like infectivity. The fibril-forming tendency of Sup35 had been traced to the amino terminus of the protein and from this region we isolated a seven-residue, fibril-forming segment with sequence GNNQQNY [1].

This peptide dissolves in water, and at a concentration of approximately 400 μM, forms amyloid-like fibrils in a few hours. These fibrils display all of the common characteristics of amyloid fibrils. When dissolved at higher concentrations (about 10–100 mM), GNNQQNY forms elongated microcrystals, no larger than ~ 40 μm in length and ~ 1 μm in diameter. Although extremely small, these crystals were well ordered, and were the first truly crystalline form of amyloid-like substances ever discovered.

The second barrier to structure elucidation was how to collect single crystal X-ray diffraction data from such small crystals. Progress came when Dr. Carl-Ivar Branden, the former Scientific Director of ESRF put the UCLA amyloid group in touch with the ESRF team who had developed the microfocus beamline **ID13**. The diffraction data collected yielded structures for GNNQQNY and for its shorter version NNQQNY [2].

The atomic structure of GNNQQNY revealed some features that were expected, and also some surprises (**Figure 78**). As expected, each GNNQQNY molecule is an extended beta chain, and each is hydrogen bonded to identical molecules above and below it forming standard parallel β-sheets. Each sheet is related to a mating sheet by a two-fold axis, and a translation along the fibril axis by one half the inter-strand separation. Surprising to us is that the two sheets are unusually close, with their side-chains intermeshed, forming what we term a 'steric

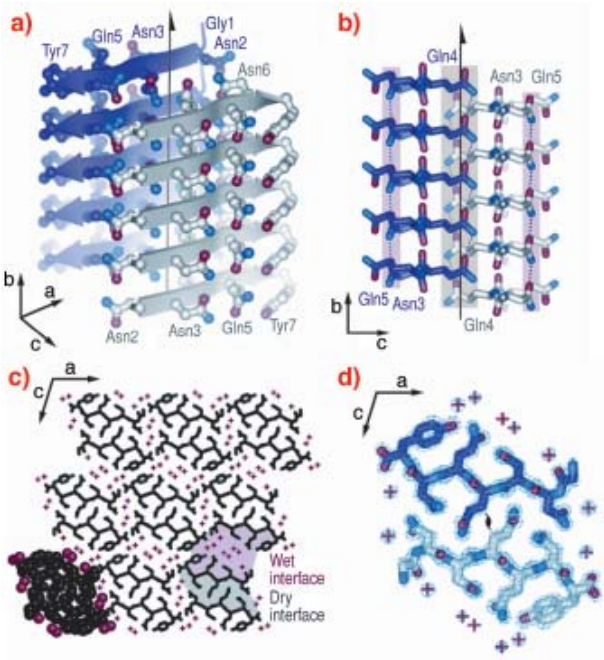

Fig. 78: Structure of GNNQQNY. Unless otherwise noted, carbon atoms are coloured in purple or grey/white, oxygen in red, and nitrogen in blue. (a) The pair-of-sheets structure showing the backbone of each β-strand as an arrow, with ball and stick sidechains protruding. (b) The steric zipper viewed down the **a** axis. The amide stacks of the dry interface are shaded in grey at the centre, and those of the wet interface are shaded in pale red on either side. (c) The GNNQQNY crystal viewed from the top of panel a, along the **b** axis. Nine pairs of β-sheets run horizontally. Peptide molecules are shown in black and water molecules are represented by red +. (d) The steric zipper: close-up view of a pair of GNNQQNY β-sheets from the same view as panel c, showing the remarkable shape complementarity of the Asn and Gln sidechains protruding into the dry interface. 2Fo-Fc electron density is shown, and the position of the central screw axis is indicated.

zipper'. This tight interface between the two sheets is utterly dry, with no water molecules and there are no hydrogen bonds from one sheet to its mate. It is this dry, closely-fitting steric zipper that holds the two sheets together. This same feature is found in microcrystals of other amyloid-forming peptides from proteins associated with human diseases that we have subsequently examined.

The biological implications of the structure of GNNQQNY are: (1) A short peptide of a few (4-7 residues), rather than an entire protein is sufficient to form amyloid-like fibrils. That is, the spine of amyloid fibril has a simpler structure than previously believed; (2) The amyloid state of protein is an unusually dry state; (3) The bond between the two sheets is extended, but formed by weak van der Waals forces. These suggest routes towards developing therapies for terrible diseases such as Alzheimer's and related amyloidoses.

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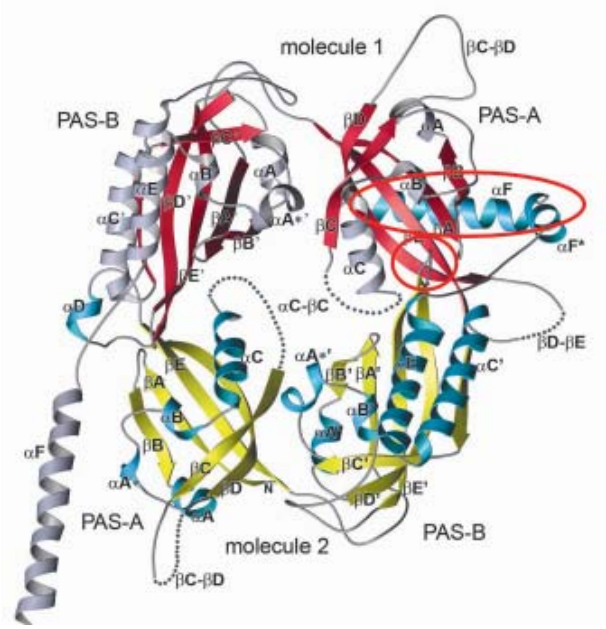

Fig. 79: Crystal structure of the *Drosophila* Period (dPER) dimer. Molecule1 is shown in red and grey, molecule2 in yellow and blue. PAS-A and PAS-B denote the two PAS domains in each monomer. The PAS-A dimer interfaces are highlighted by red circles.

Crystal Structure of Drosophila Period Protein: Insight into Circadian Clock Regulation

Most organisms exhibit 24-hour activity cycles called *circadian rhythms* (from the latin *circa diem* = about a day). Circadian rhythms are generated by circadian clocks, which are operated by interconnected transcriptional-translational feedback loops [1]. Period (PER) proteins are central components of the *Drosophila* and mammalian circadian clocks. Their function is controlled by daily changes in synthesis and degradation, cellular localisation, phosphorylation as well as specific interactions with other clock components. In order to shed some light on these molecular mechanisms, we have solved the crystal structure of a *Drosophila* Period (dPER) fragment comprising two tandemly-organised PAS (PER-ARNT-SIM) domains (PAS-A and PAS-B) and two additional C-terminal α-helices (αE and αF). To this end, we have collected 3.5 Å SeMet-MAD data for experimental phasing at beamline **ID29** and a 3.15 Å native data set for final refinement at beamline **ID14-2.**

PAS domains represent a diverse and ubiquitous family of sensory-, signalling- and protein-protein interaction modules, which consist of a five-stranded antiparallel ß-sheet (ßA-ßE) covered on one face by several α-helices (αA-αC). The crystal structure of the dPER PAS repeat region reveals a non-crystallographic dimer stabilised by two sets of intermolecular PAS domain interactions (**Figure 79**). The dimer interfaces are mediated by interactions of PAS-A with a conserved tryptophan residue, Trp482, in the ßD'-ßE'-loop of PAS-B (PAS-A-Trp interface, **Figure 79** and **Figure 80a**) and with the helix αF located at the C-terminal to PAS-B (PAS-A-αF interface, **Figures 79, 80b**), respectively. Interestingly, a point mutation which leads to extended (29 hour) days in living flies (*per*^{L} mutation) is located in the centre of the PAS-A-αF interface. The *per*^{L} mutation, which corresponds to an exchange of Val243 to Asp

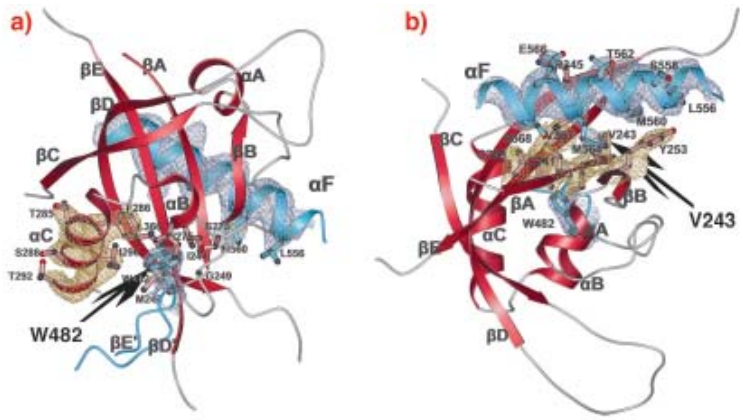

Fig. 80: PAS-A dimer interfaces of dPER. The PAS-A domain of molecule 1 is shown in red. Helix αF and the PAS-B-ßD'-ßE' loop with Trp482 of molecule 2 are shown in blue. The two PAS-A orientations provide closeup views of (a) the PAS-A-Trp482 dimer interface and (b) the PAS-A-αF dimer interface. W482 and the *per*^{L} mutation site V243 are labelled at the respective interfaces.