ESRF

ESRF	**Experiment title:** Structural basis of Pdcd4 modulating the translation initiation activity of eIF4A	**Experiment number**: MX-752
Beamline: ID14 4	**Date of experiment**: from: 07/05/2008 to: 08/05/2008	**Date of report**: Feb. 19, 2010
Shifts: 2	**Local contact(s)**: Dr. Dominique BOURGEOIS	*Received at ESRF:*

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Report:

Pdcd4 is a tumor suppressor protein. It inhibits translation through interaction with translation initiator eIF4A, resulting in the suppression of neoplastic transformation and tumor invasion. Here, we present the crystal structures of an N-terminal truncated Pdcd4 in free form and in complex with eIF4A. Upon binding to eIF4A, Pdcd4 undergoes a marked conformational change to form a heterotrimeric complex with eIF4A, with one Pdcd4 binding to two eIF4A molecules in two different modes. The binding of Pdcd4 to eIF4A is required to inhibit the enzymatic activity of eIF4A, translation initiation, and AP-1-dependent transcription. Both MA3 domains are required to efficiently compete with the C-terminal domain of eIF4G (eIF4Gc) for binding to eIF4A while a single MA3 is sufficient to inhibit translation. Our structural and mutational analyses reveal that Pdcd4 inhibits translation initiation by trapping eIF4A in an inactive conformation, and blocking its incorporation into the eIF4F complex.

Table 1. Data collection and refinement statistics

Data collection	Se-Met hPdcd4ΔN	mPdcd4ΔN-eIF4A
Wavelength (Å)	0.9798	0.9725
Resolution limit (Å)	2.87	3.5
Space group	$P3_121$	$P2_13$
Unit cell dimensions		
a, b, c (Å)	170.33, 170.33, 66.92	198.36, 198.36, 198.36
α, β, γ (˚)	90, 90, 120	90, 90, 90
Unique reflections (N)	27711	32991
I/σ	5.0 (2.1)	5.8 (1.9)
Completeness (%)	100.0 (100.0)	99.8 (100.0)
Redundancy	9.8(9.4)	11.9 (12.2)
R_{merge} [a]	0.084 (0.435)	0.108 (0.399)
Figure of merit		
Before density modification	0.152	
After density modification	0.815	

Refinement		
Resolution range (Å)	20 – 2.87	30 – 3.5
Used reflections (N)	26320	31302
Nonhydrogen atoms (water)	4426 (56)	8034 (–)
R_{work} [b] (%) / R_{free} [c] (%)	23.1 / 27.3	25.0 / 29.0
R.m.s. deviations		
Bond lengths (Å)	0.007	0.007
Bond angles (˚)	1.069	1.146
Ramachandran plot		
Allowed (% residues)	99.0	98.7
Generously allowed (% residues)	1.0	1.1
Disallowed (% residues)	0.0	0.2

Values in parentheses indicate the specific values in the highest resolution shell.

[a] $R_{merge} = \Sigma|I_j - \langle I \rangle|/\Sigma I_j$, where I_j is the intensity of an individual reflection, and $\langle I \rangle$ is the average intensity of that reflection.

[b] $R_{work} = \Sigma||F_o| - |F_c||/\Sigma |F_c|$, where F_o denotes the observed structure factor amplitude, and F_c denotes the structure factor amplitude calculated from the model.

[c] R_{free} is as for R_{work} but calculated with 5.0% of randomly chosen reflections omitted from the refinement.

Reference:

Loh PG, Yang HS, Walsh MA, Wang Q, Wang X, Cheng Z, Liu D, Song H. (2009). Structural basis for translational inhibition by the tumour suppressor Pdcd4. *EMBO J*. 28, 274-285.