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The MRG domain of human MRG15 uses a shallow hydrophobic pocket to interact with the N-terminal region of PAM14

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Introduction

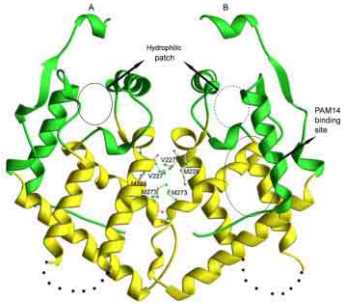
MRG15 is a transcription factor expressed in a variety of human tissues and its orthologues have been found in many other eukaryotes which constitute the MRG protein family [1]. It plays a vital role in embryonic development and cell proliferation and is involved in cellular senescence. The C-terminal part of MRG15 forms a conserved MRG domain which is involved in interactions with the tumor suppressor protein retinoblastoma and a nucleoprotein PAM14 during transcriptional regulation [2]. We report here the crystal structure of the MRG domain of human MRG15 (MRG15C) at 2.2 Å resolution and the characterization of its interaction with PAM14 using both yeast two-hybrid and *in vitro* binding assays. The MRG domain is predominantly hydrophobic and assumes a new protein fold consisting of mainly α-helices that are arranged in a three-layer sandwich topology. Structure-based site-directed mutagenesis studies identified key residues involved in the binding of N-terminal of PAM14.

Materials and Methods

The cDNA corresponding to MRG15C (residues 151-323) was cloned into the pET-28a(+) and the protein was induced and purified. Crystallization of MRG15C was carried out using the hanging drop vapor diffusion method. Crystals of both native and Se-Met MRG15C were grown in drops containing equal volumes of the protein solution (20 mg/ml) and the crystallization solution (0.1 M HEPES, pH 7.9, 20% PEG4000, and 5% iso-propanol) to approximate dimensions of 0.2 x 0.2 x

0.3 mm3 in 10 days at 20 °C. Crystals of MRG15C belong to space group R3 with the cell parameters of a=b=111.2 Å and c=87.0 Å. The native diffraction data were collected to 2.2 Å resolution from a flash-cooled crystal at 100 K at beamline BL-6A of Photon Factory, Japan. The multi-wavelength anomalous dispersion (MAD) data were collected to 2.6 Å resolution at beamline BL-18B of Photon Factory. Crystal structure of MRG15C was solved by the MAD method.

Structure analysis and in-vitro binding assay reveal a hydrophobic pocket to interact with the N-terminal of PAM14 through primarily hydrophobic interactions. The paper will be published in the near future.



References

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