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Letter to the Editor

A Sliding Clamp Model for the Rad1 Family of Cell Cycle Checkpoint Proteins

We have identified an unexpected structural similarity between six members of the Rad1 cell cycle checkpoint family and the DNA sliding clamp protein PCNA. Like its prokaryotic structural homolog, the β subunit of DNA polymerase III, PCNA facilitates genome replication by encircling the DNA helix and tethering DNA polymerase to its substrate. Our Rad1 model predicts that eukaryotic cells contain a second PCNA-like structure, one that may be critical to the mechanisms coupling DNA repair and DNA synthesis to a mitotic checkpoint.

The Rad1 family of proteins, including Rec1 of *Ustilago maydis* and its distant relatives Rad1^{sp} (*S. pombe*) and Rad17^{sc} (*S. cerevisiae*), function in both DNA repair and cell cycle control (Lydall and Weinert, 1995; Onel et al., 1996). Homologs have been found in fly (M. Brodsky, M. P. T., and G. Rubin, unpublished data), mouse, and human (Bluyssen et al., 1998; Dean et al., 1998; Freire et al., 1998; Marathi et al., 1998; Parker et al., 1998a; Udell et al., 1998). Sequence comparisons reveal conserved blocks of amino acids between these six proteins, but overall there is less than 30% identity between any two sequences. Similarity to other known proteins is not obvious, and a common biochemical function remains unclear.

We explored the possibility that the Rad1 family members have more distant homology with other proteins of known function. To do this, six conserved blocks in the Rad1 family were embedded into the Rad1^{Mm} sequence using BLOCKs, and this cobbled sequence was used as the probe in a PSI-BLAST search. An initial weak match was amplified after several iterations, and PCNA (*S. cerevisiae*, and other species) was retrieved as a candidate homolog with nearly end-to-end sequence alignment. However, PCNA and the probe sequence were only 15% identical, making it uncertain whether these protein families are truly related.

We therefore sought independent verification of this hypothesis using the methods of fold recognition and comparative modeling. Using an empirically derived fitness function, fold recognition (threading) evaluates the compatibility of a new sequence with templates in the library of known folds. Threading was performed for each of the six homologs separately. In each case, only the PCNA fold was always among the top ranking hits. More importantly, for the Rad1^{Mm}, Rad1^{Dm}, and Rad17^{Sc} sequences, threading assigned PCNA as the number one structure, with scores of 11.8, 11.8, and 8.1, respectively, all of which were well above the independently established false positive threshold (Fischer and Eisenberg, 1996).

The hypothesis was further explored by building and evaluating an all atom model of Rad1^{Mm}. Using the PSI-BLAST alignment as a guide, Rad1^{Mm} amino acid side chains were placed on the peptide backbone of the

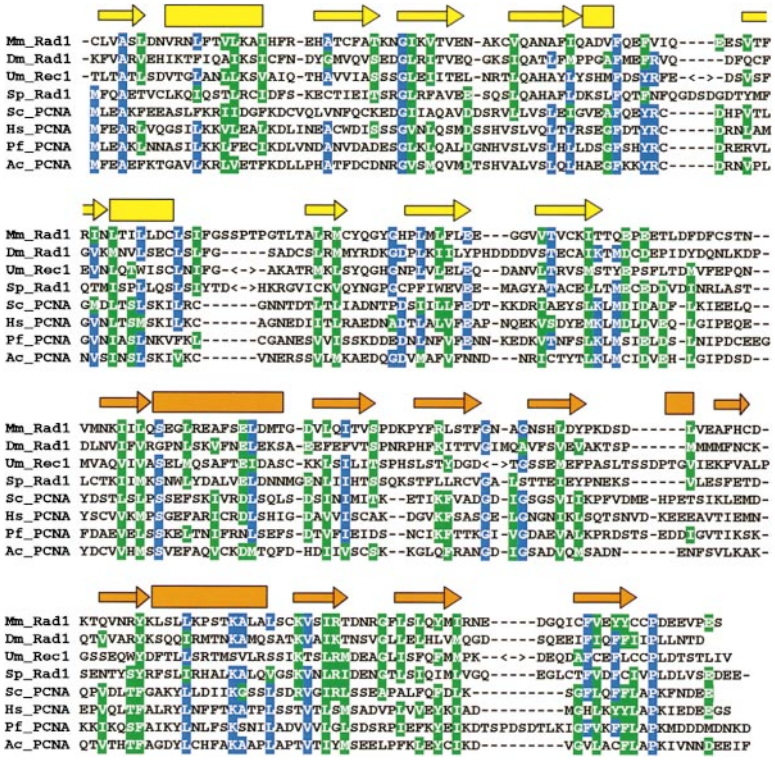

Figure 1. Multiple Sequence Alignment of Rad1 and PCNA Family Members

Rad1 homologs include those from mouse (Mm_Rad1), fruit fly (Dm_Rad1), smut fungus (Um_Rec1), and fission yeast (Sp_Rad1). PCNA sequences are from budding yeast (Sc_PCNA), malaria parasite (Pf_PCNA), baculovirus (Ac_PCNA), and human (Hs_PCNA). Rad1 family sequence alignment with PCNA proteins relied on Rad1^{Mm} modeling studies that used PCNA^{Sc} as a structural template. <–>, regions removed from the Um_Rec1 and Sp_Rad1 sequences to make the alignment more compact. Short unaligned N-terminal (Mm_Rad1 and Um_Rec1) and C-terminal (Sp_Rad1) regions were also excluded. Residues conserved in more than half the sequences are colored green (identical) and blue (similar). Secondary structure of Sc_PCNA is shown as arrows (strands) and rectangles (helices); different colors represent two distinct domains. Mm_Rad1 and Sc_PCNA are 13.5% identical in the final alignment.

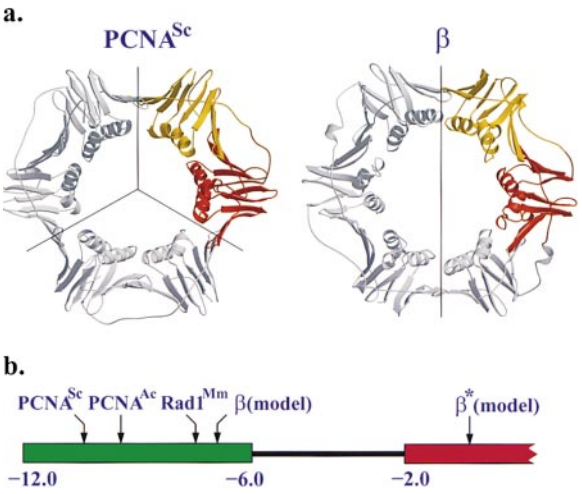

Figure 2. Comparative Modeling of Rad1 and PCNA Homologs

(a) Molecular structures of PCNA and β subunit of DNA polymerase III. Line segments indicate unique polypeptide chains of the DNA clamp assemblies (crystal structure PDB codes 1plq and 2pol, respectively). Coloring of the two structural domains of the PCNA monomer (yellow and orange) reflects the secondary structure assignment shown in Figure 1. Corresponding structural domains of β are shown in like colors. The sequence of these two domains was used to build models of β and β* using the PCNA template (ProsaII evaluation, Figure 2B). MOLSCRIPT and Raster3D were used to generate the structural representations.

(b) Evaluation of comparative modeling. Results of ProsaII structure assessments for the models of (1) $Rad1^{Mm}$, (2) viral $PCNA^{Ac}$, a distant but highly conserved ortholog of $PCNA^{Sc}$, (3) β, the sliding clamp subunit of DNA polymerase III (Kong et al., 1992), and (4) the crystal structure of $PCNA^{Sc}$ (Krishna et al., 1994). Green segment denotes region typically occupied by native structures of soluble globular proteins of approximately the same size as the $PCNA^{Sc}$ monomer, and red, erroneous or deliberately misfolded structures. ProsaII places our $Rad1^{Mm}$ model structure between the models of $PCNA^{Ac}$ and β, for which the correctness of the PCNA fold is certain. Sensitivity of ProsaII evaluations is illustrated by a comparison with another model of β, one generated using the same template but with an incorrect alignment (Kong et al., 1992). ProsaII identified the model based on the incorrect alignment (β*) as an incorrect structure. Respective ProsaII Z scores (Sippl, 1993) are −10.42 for $PCNA^{Sc}$, −9.45 for $PCNA^{Ac}$, −7.36 for $Rad1^{Mm}$, −6.81 for β, and −0.36 for β*.

$PCNA^{Sc}$ template. The model structure was then optimized by adjusting the sequence alignment and conformation of the side chains to alleviate steric clashes or unfavorable electrostatic interactions. The resulting sequence-to-structure alignment was the basis for comparing several members of the two families of proteins (Figure 1). Quality of the $Rad1^{Mm}$ model was assessed with ProsaII, a method designed to detect errors in the 3D structure of proteins (Sippl, 1993). Results of this assessment are very similar to those found for error-free experimental structures. Additional valuable reference points are provided by modeling related proteins, with the same structure of PCNA as a template (Figure 2). The results of threading and comparative modeling provide complementary and compelling evidence that PCNA is a correct structural template for the Rad1 homologs.

The sliding clamp model for Rad1 proteins is further strengthened by evidence from protein–protein interaction analyses. The known sliding clamp proteins, PCNA and β, are actively loaded onto DNA by the RFC and γ protein complexes, respectively. Proteins homologous to these clamp loaders (Griffiths et al., 1995) may also interact specifically with Rad1 family members; for example, $Rad1^{Hs}$ interacts with $Rad17^{Hs}$ (Parker et al., 1998b). This combined homology information indicates that a biochemical function for Rad1 homologs might resemble or augment PCNA, providing similar clamp-like processivity for DNA repair and synthesis enzymes. However, due to the large degree of sequence divergence between these proteins, it is expected that differences from PCNA structure will be found, perhaps to accommodate the 3′→5′ exonuclease activity reported for Rad1 homologs (Thelen et al., 1994; Parker et al., 1998a).

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References

Bluyssen, H.A., van Os, R.I., Naus, N.C., Jaspers, I., Hoeijmakers, J.H., and de Klein, A. (1998). Genomics *54*, 331–337.

Dean, F.B., Lian, L., and O'Donnell, M. (1998). Genomics *54*, 424–436.

Fischer, D., and Eisenberg, D. (1996). Protein Sci. *5*, 947–955.

Freire, R., Murguia, J.R., Tarsounas, M., Lowndes, N.F., Moenes, P.B., and Jackson, S.P. (1998). Genes Dev. *15*, 2560–2573.

Griffiths, D.J., Barbet, N.C., McCready, S., Lehmann, A.R., and Carr, A.M. (1995). EMBO J. *14*, 5812–5823.

Kong, X.P., Onrust, R., O'Donnell, M., and Kuriyan, J. (1992). Cell *69*, 425–437.

Krishna, T.S., Kong, X.P., Gary, S., Burgers, P.M., and Kuriyan, J. (1994). Cell *79*, 1233–1243.

Lydall, D., and Weinert, T. (1995). Science *270*, 1488–1491.

Marathi, U.K., Dahlen, M., Sunnerhagen, P., Romero, A.V., Ramagli, L.S., Siciliano, M.J., Li, L., and Legerski, R.J. (1998). Genomics *54*, 344–347.

Onel, K., Koff, A., Bennett, R.L., Unrau, P., and Holloman, W.K. (1996). Genetics *143*, 165–174.

Parker, A.E., Van de Weyer, I., Laus, M.C., Oostveen, I., Yon, J., Verhasselt, P., and Luyten, W.H. (1998a). J. Biol. Chem. *273*, 18332–18339.

Parker, A.E., Van de Weyer, I., Laus, M.C., Verhasselt, P., and Luyten, W.H. (1998b). J. Biol. Chem. *273*, 18340–18346.

Sippl, M.J. (1993). Proteins *17*, 355–362.

Thelen, M.P., Onel, K., and Holloman, W.K. (1994). J. Biol. Chem. *269*, 747–754.

Udell, C.M., Lee, S.K., and Davey, S. (1998). Nucleic Acids Res. *26*, 3971–3976.