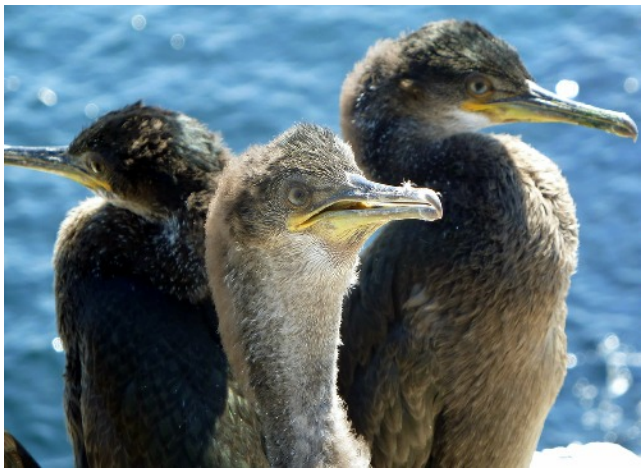
Are we really related?

The Rad9/Ddc1 family

By Hsin-Yu Chang



Holiday season is approaching! During the holidays, presents are exchanged, meals are shared, parties are enjoyed. It is the time when families get together to catch up on each other's lives. The Chinese character '家 (family or home)' is derived from an ancient ideogrammic compound word consisting of a pig '豕' under a roof '宀', because pigs formerly lived in Chinese houses. The definition of a family has changed dramatically since. Pigs no longer live under people's roofs and families are usually composed of a few nuclear family members. In biology, ways of thinking about protein families have also evolved over the years. With high quality genome and protein sequencing data, it is possible to obtain protein family classifications using computerised multiple protein sequence alignment and structural analysis. Nevertheless, protein family classifications need to be inspected carefully in order to get the full picture. Let's have a look at an example – the Rad9/Ddc1 family.

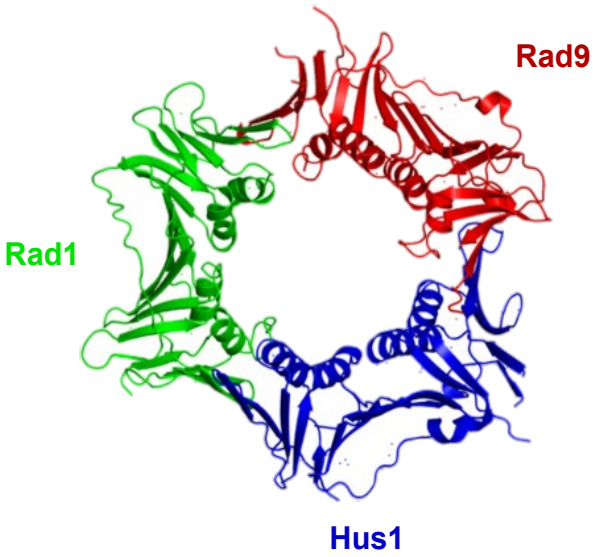


Figure 1. The structure of the human 9-1-1 complex from PDB (3g65).

The Rad9 gene was first isolated from fission yeast (*S. pombe*) by complementing the gamma-ray sensitivity of the mutant cell line [1,2]. Subsequently, a few homologues were identified from different species, such as other yeasts, mouse and human. Human Rad9 forms a protein complex with Rad1 and Hus1 proteins, called the 9-1-1 complex. This complex forms a ring-shaped structure (Figure 1) and plays an important role in DNA repair responding to DNA damage [3,4]. Interestingly, in budding yeast (*S. cerevisiae*), another protein, Ddc1, shows weak homology to the human Rad9 and forms a similar ring structure (with Rad17 and Mec3), which has similar functions to the 9-1-1 complex [5]. So, Rad9 and Ddc1 appear to be related. But can we say that they belong to the same family?

Aligning the protein sequences of a few Ddc1 and Rad9 family members from UniProt shows that the Rad9 members in human, mouse and fission yeast are more similar to each other than to the Ddc1 members from the budding yeast (Figure 2).

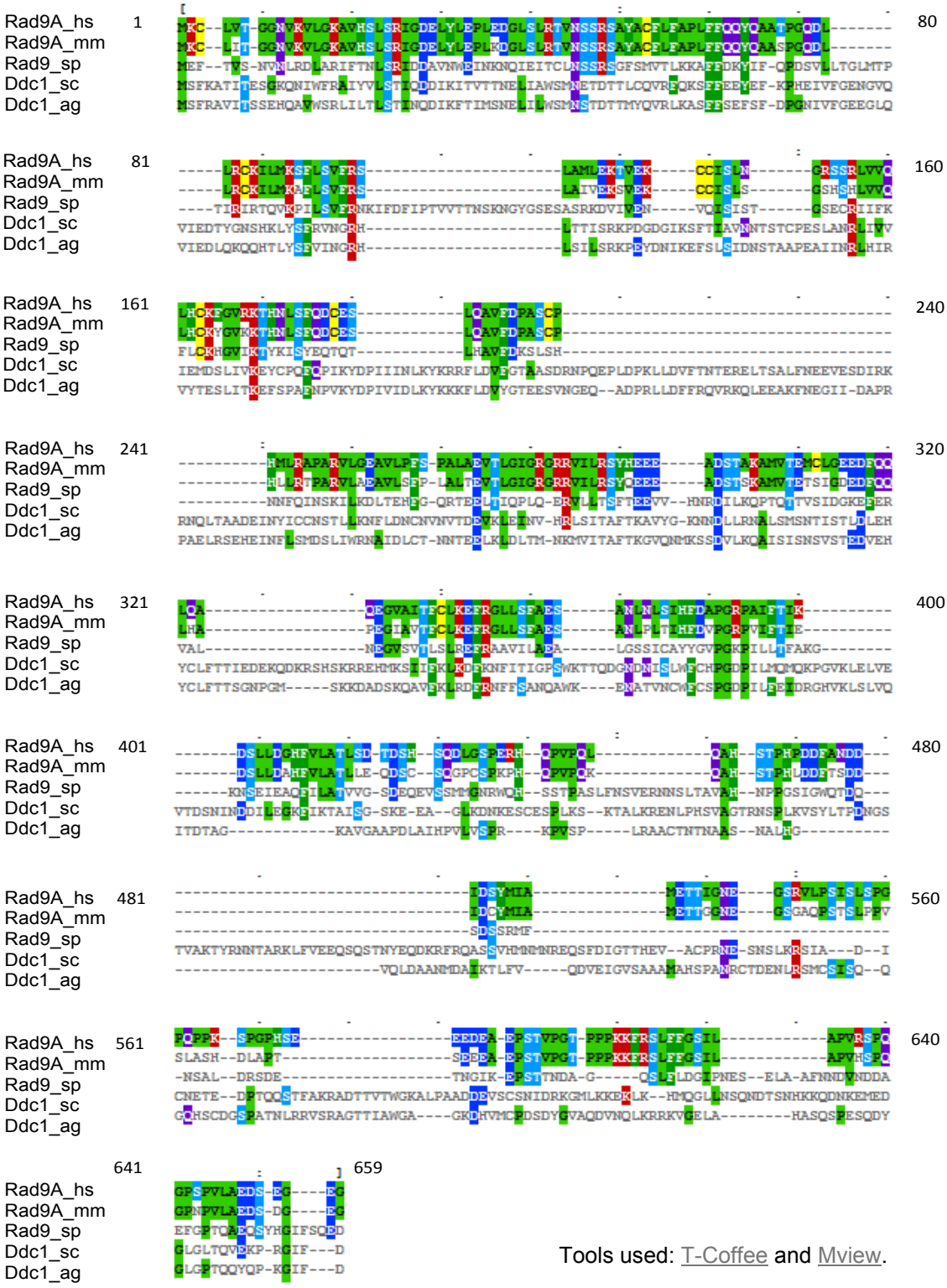


Figure 2. Multiple protein sequence alignment of the Ddc1 and Rad9 members. sc: *Saccharomyces cerevisiae* (budding yeast), ag: *Ashbya gossypii* (budding yeast), hs: *Homo sapiens* (human), mm: *Mus musculus* (mouse), sp: *Schizosaccharomyces pombe* (fission yeast).

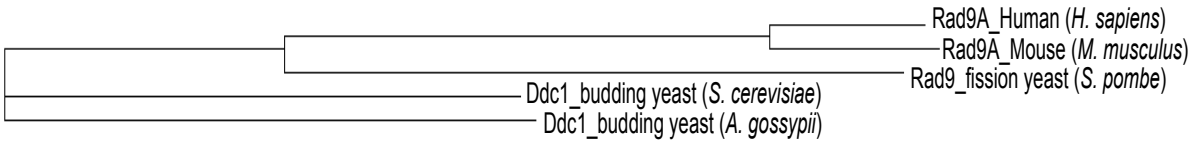


Figure 3. Phylogram of the Ddc1 and Rad9 members.

Using alignment data, a phylogenetic tree can be drawn, allowing us to infer evolutionary relationships between the proteins (Figure 3). It's clear that the *S. pombe* node lies between the human/mouse and budding yeast clades.

Using the InterPro protein classification database, we can further investigate the relationship between the Rad9/Ddc1 families. Searching the database with the keyword "Rad9" returns three relevant entries: Rad9/Ddc1 (IPR007268), Rad9 (IPR026584), and Ddc1 (IPR026217). We summarise the computational methods used to construct these entries, the number of protein sequences they match and taxonomic information in Table 1.

Interestingly, Ddc1 family members only appear to be found in budding yeasts, while Rad9* family members can be found in other non-budding-yeast fungi and eukaryotes. Meanwhile, the entry IPR007268 matches both Rad9 and Ddc1 family members and groups them into a larger family (see Figure 4).

InterPro ID	Entry name	Signatures	UniProt Proteins matched	Species
IPR007268	Rad9/Ddc1	PF04139 (Pfam)	~317	Eukaryotes (including *Saccharomycetaceae*)
IPR026584	Rad9	PTHR15237, (PANTHER) PIRSF009303 (PIRSF)	~240	Eukaryotes (Including fungi which are non *Saccharomycetaceae*)
IPR026217	Ddc1	PR02063 (PRINTS)	~27	Fungi *Saccharomycetaceae* (budding yeasts)

Table 1. The comparison of the InterPro entries representing Rad9/Ddc1, Rad9 and Ddc1 families.

***It's worth noting that the name 'Rad9' conflicts with a DNA damage-dependent checkpoint protein in *S. cerevisiae*. In this article, we refer Rad9 as the 'Rad9' in human 9-1-1 complex and its homologues.**

From the literature, we know that Rad9 and Ddc1 are more similar at the protein structural level than at the protein sequence level [3]. This represents an example of evolutionary divergence in different species resulting in many variants of the same protein with similar structure and biological functions [6]. Interestingly, structural similarity is also shared between the 9-1-1 and the Ddc1-Rad17-Mec3 complexes. All three of the subunits: Rad9 and Ddc1 (IPR007268), Rad1 and Rad17 (IPR003021), Hus1 and Mec3 (IPR007150) structurally resemble each other. It has been suggested that proteins from these complexes evolved from a common ancestor [4, 7].

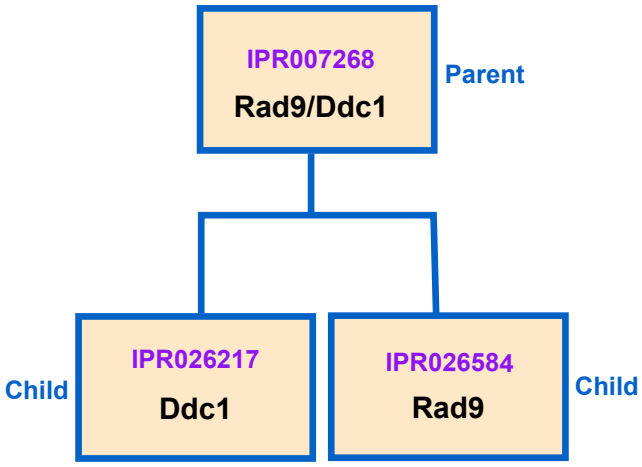


Figure 4. Relationships between Rad9/Ddc1, Rad9 and Ddc1 families.

To define a protein family is not always straightforward. So far, we have seen that Ddc1 and Rad9 can be classified as belonging to the same family. However, by using more specific methods, they can also be separated into two different families, with Ddc1 members from budding yeasts in one and Rad9 members from all eukaryotes excluding budding yeasts in the other. These different levels of classification address different aspects of protein evolution and function. Grouping the proteins into the larger Rad9/Ddc1 family can help us to examine their similarities and allow us infer information about their common ancestor. Grouping the proteins into smaller families (Rad9 and Ddc1) allows us to distinguish their separate functions - for instance, some Rad9 orthologues possess DNA exonuclease function, while Ddc1 does not [8,9]. As with human society, what makes a family can not be precisely defined. What is important is that by analysing them, we can understand individuals and communities a bit better.

References

1. Murray JM, Carr AM, Lehmann AR, Watts FZ. Cloning and characterisation of the rad9 DNA repair gene from Schizosaccharomyces pombe. Nucleic Acids Res. 19(13):3525-31.1991 [PubMed: 1852603].
2. Lieberman HB, Hopkins KM, Laverty M, Chu HM. Molecular cloning and analysis of Schizosaccharomyces pombe rad9, a gene involved in DNA repair and mutagenesis. Mol Gen Genet. 232(3):367-76. 1992 [PubMed: 1588907].
3. Doré AS, Kilkenny ML, Rzechorzek NJ, Pearl LH. Crystal structure of the rad9-rad1-hus1 DNA damage checkpoint complex-implications for clamp loading and regulation. Mol Cell. 34(6):735-45. 2009 [PubMed:19446481].

4. Sohn SY, Cho Y. Crystal structure of the human rad9-hus1-rad1 clamp. J Mol Biol. 390(3):490-502. 2009 [PubMed: 19464297].
5. Kondo T, Matsumoto K, Sugimoto K. Role of a complex containing Rad17, Mec3, and Ddc1 in the yeast DNA damage checkpoint pathway. Mol Cell Biol. 19(2):1136-43. 1999 [PubMed: 9891048].
6. Patthy L (1999). Protein evolution. United Kingdom, Blackwell Science.
7. Venclovas C, Thelen MP. Structure-based predictions of Rad1, Rad9, Hus1 and Rad17 participation in sliding clamp and clamp-loading complexes. Nucleic Acids Res. 28(13):2481-93. 2000 [PubMed:10871397].
8. Bessho T, Sancar A. Human DNA damage checkpoint protein hRAD9 is a 3' to 5' exonuclease. J. Biol. Chem. 275(11):7451-7454. 2000 [PubMed: 10713044].
9. Majka J, Burgers PM. Yeast Rad17/Mec3/Ddc1: a sliding clamp for the DNA damage checkpoint. Proc. Natl. Acad. Sci. U.S.A. 100(5):2249-54. 2003 [PubMed: 12604797] .

Useful Links

InterPro
http://www.ebi.ac.uk/interpro/

Rad9/Ddc1 family (InterPro)
http://www.ebi.ac.uk/interpro/search?q=IPR007268

Rad9 family
http://www.ebi.ac.uk/interpro/search?q=IPR026584

Ddc1 family
http://www.ebi.ac.uk/interpro/search?q=IPR026217

UniProt
http://www.uniprot.org

Crystal structure of the Human Rad9-Rad1-Hus1 DNA damage Checkpoint Complex (PDB)
http://www.ebi.ac.uk/pdbe-srv/view/entry/3g65/summary

T-Coffee - Multiple Sequence Alignment
http://www.ebi.ac.uk/Tools/msa/tcoffee/

MView - A multiple alignment viewer
http://www.ebi.ac.uk/Tools/msa/mview/

ClustalW2 - Phylogeny
http://www.ebi.ac.uk/Tools/phylogeny/clustalw2_phylogeny/

Let us know

If you have any comments on this article please contact:
interhelp@ebi.ac.uk