Published in final edited form as:
Gene. 2011 October 15; 486(1-2): 23–30. doi:10.1016/j.gene.2011.06.030.

The Evolution of *MDM2* family genes

Jamil Momand[a], **Alberto Villegas**[a], and **Vladimir A. Belyi**[b,c]

Jamil Momand: jmomand@calstatela.edu; Alberto Villegas: vbelyi@ias.edu

[a]Department of Chemistry and Biochemistry, California State University Los Angeles, Los Angeles, CA 90032 US

[b]Institute for Advanced Study, Princeton, New Jersey 08540 US

[c]New affiliation: Cancer Institute of New Jersey, University of Medicine and Dentistry of New Jersey, New Brunswick, NJ 08901

Abstract

MDM2 and MDM4 are proto-oncoproteins that bind to and inhibit members of the p53 protein family, p53, p73 and possibly p63. p53 is a mammalian tumor suppressor and p63 and p73 are critical for development. With the sequencing of genomes from multiple organisms there is mounting evidence for a consensus scenario of *p53* gene family evolution. A single *p53/p63/p73* gene is in invertebrates and required for maintenance of germline DNA. Gene duplication occurred in an ancestor in common with cartilaginous fishes, giving rise to a separate *p53* gene and at least one ancestral *p63/p73* gene. In bony vertebrates, all three *p53* gene family paralogs, *p53*, *p63*, and *p73* are distinct genes. This raises the question of how *MDM2* and *MDM4* genes evolved. We show evidence that *MDM2* and *MDM4* arose from a gene duplication event prior to the emergence of bony vertebrates more than 440 million years ago. Comparative genome studies indicate that invertebrate organisms have only one *MDM* homolog. In jawed vertebrates, the p53-binding domains of MDM2 and MDM4 proteins evolved at a high rate, approaching the evolution rate of the MDM2-binding domain of p53. However, the MDM2-binding domain of p73 exhibits markedly stronger conservation suggesting novel p53-independent functions. The most conserved domain within all MDM2 family members is the RING domain of the MDM2 ortholog which is responsible for ubiquitination of p53 and heterodimerization with MDM4. We suggest a model where dimerization is an ancient function of MDM and ubiquitination activity was acquired later near the *MDM* gene duplication event coinciding with the time of the emergence of *p53* as a distinct gene.

Keywords

cancer; MDM; MDM2; MDM4; MDMX; p53

Corresponding author: Jamil Momand, Dept of Chemistry and Biochemistry, California State University Los Angeles, 5151 State University Drive, Los Angeles, CA 90032 US, Tel: 001-323-343-2361, Fax: 001-323-343-6490, jmomand@calstatela.edu.

1.0 Introduction

MDM2 is a proto-oncoprotein and its gene is located on human chromosome 12q14.3–q15. The *MDM2* gene was first discovered in double minutes isolated from the tumorigenic mouse cell line 3T3DM (Cahilly-Snyder et al., 1987; Fakharzadeh et al., 1991). Double minutes are small extrachromosomal DNAs that arise from gene amplifications. The *MDM2* gene is amplified in 7 percent of all human cancers with greater frequencies in soft tissue tumors, osteosarcomas, and esophageal carcinomas (Oliner et al., 1992; Momand et al., 1998). In cancers with no apparent *MDM2* amplification, MDM2 transcript levels can be elevated by increased expression from *MDM2* promoter elements responsive to Smad3/4 and SP1 (Bond et al., 2006; Araki et al., 2010). MDM2 is an E3 ubiquitin ligase that mediates polyubiquitination of p53, tagging it for degradation by the 26S proteasome (Haupt et al., 1997; Honda et al., 1997; Kubbutat et al., 1997). Genetic and biochemical data compiled over the past 20 years show that the primary function of MDM2 is to inhibit p53 tumor suppressor activity (Wade et al., 2010).

MDM4 (also known as MDMX) is a paralog of MDM2, discovered by screening a mouse cDNA expression library with radiolabeled p53 protein (Shvarts et al., 1996). The *MDM4* gene is located on human chromosome 1q32 and is amplified in brain/nervous tissue cancers, breast cancers, and soft tissue tumors at a frequency of 10–25 percent (Toledo and Wahl, 2006; Liang et al., 2010). MDM4 protein levels are elevated in at least 17 percent of mantle cell lymphomas, breast cancers, uterine cancers, testicular cancers, stomach/small intestinal cancers, colorectal cancers, lung cancers, and malignant melanomas. *MDM4* DNA copy number is increased in 65% of human retinoblastomas (Laurie et al., 2006). MDM2 and MDM4 have similar patterns of protein domain organization. Both contain a p53 binding domain, an acidic domain, a zinc finger and a RING domain. In humans, MDM2 and MDM4 share 31 percent amino acid identity over their entire coding sequences. The two proteins form heterodimers through their RING domains and MDM4 stimulates MDM2-mediated polyubiquitination of p53 (Linares et al., 2003).

The target of MDM2 and MDM4 downregulation, p53, is part of a family of three paralogs that includes two other transcription factors, p63 and p73. MDM2 has been shown to bind to and inhibit p53-and p73-mediated transactivation (Momand et al., 1992; Balint and Reisman, 1996), although MDM2 does not ubiquitinate p73 (Zeng et al., 1999). Evidence for MDM2 and MDM4 binding to p63 *in vivo* is not clear (Kadakia et al., 2001; Kojima et al., 2001; Little and Jochemsen, 2001; Wang et al., 2001; Calabro et al., 2002). Dissociation constant measurements show that MDM2 and MDM4 binding to p63 is ten-fold weaker than binding to p53 and p73 (Zdzalik et al., 2010). All three p53 family proteins are transcription factors that directly bind to DNA elements consisting of two copies of the 10 base pair motif 5'-PuPuPuC(A/T)(T/A)GPyPyPy-3' separated by 0–13 base pairs (el-Deiry et al., 1993; Brandt et al., 2009). The DNA binding domains of p53, p63 and p73 are similar in sequence and structure.

Insights into the distinct functions of each p53 family member have recently been gained from analysis of their genes in non-primate organisms. All three p53 family members are found in virtually all jawed vertebrates (Euteleostomi) (Belyi et al., 2010). Most multicellular species other than jawed vertebrates retain at least one p53 family member, and there is even evidence for p53 family homologs in single celled eukaryotes (Rutkowski et al., 2010). It has been suggested that in primitive multicellular organisms, a single *p53/p63/p73* gene may have first evolved to protect germ-line gametes from DNA damage and that in the early vertebrates, starting with cartilaginous fish, the *p53* gene diverged from a *p63/p73* ancestor to specialize in guarding the somatic cell genome from DNA damage (Belyi et al., 2010). Since MDM2 and MDM4 regulate p53 and p73, one might expect the MDM2 family

and p53 family genes to be consistently detected in the same species. This is clearly not the case as Drosophila melanogaster and Caenorhabditis elegans (C. elegans) express p53 family genes, but no MDM2 family members have yet been found in these organisms. In C. elegans, *ape-1* (homolog of human iASPP) is the major inhibitor of *cep-1* (homolog of human p53) (Bergamaschi et al., 2003).

In this study we report on the evolution of MDM2 and MDM4. We show evidence for MDM2 and MDM4 paralogs in jawed vertebrates, but only single MDM1 homologs in invertebrates. We present data for the existence of at least seven invertebrate MDM homologs, some of which were previously reported (Lane et al., 2010a; Lane et al., 2010b; Muttray et al., 2010). The invertebrate MDM homologs are in sea squirt (C. intestinalis), Florida lancelet (B. floridae), owl limpet (L. gigantean), acorn worm (S. kowalevskii), bay mussel (M. trossulus), deer tick (I. scapularis), and placozoan (T. adhaerens). We also show that individual domains of MDM2 and MDM4 had markedly different evolution rates, emphasizing their specialization.

2.0 Methods

2.1 Sequence identification

Jawed vertebrate and the invertebrate sea squirt C. intestinalis orthologs of human MDM2 were obtained from Ensembl genome database (Flicek et al., 2011). See Table S1 (supplemental data) for list of vertebrate MDM2 and MDM4 sequences used in this study. Six additional invertebrate MDM sequences were identified either from PsiBLAST analysis with human MDM2 or from previously published data. These were Florida lancelet, bay mussel, owl limpet, deer tick, placozoans and acorn worm. We identified three p53 family member homologs in acorn worm (GenBank IDs: XP_002732135, XP_002738810, NP_001161624). The first homolog was identified through PsiBLAST analysis of the invertebrate sequences in GenBank with human p53 sequence. The second and third sequences were identified by searching GenBank acorn worm sequences with sequence XP_002732135.

Human MDM2 sequence was used in a PsiBLAST search of invertebrate sequences in GenBank. Other invertebrate sequences similar to MDM2 were identified, but did not pass our quality criteria. These sequences are from sea anemone (N. vectensis GenBank ID: XM_001637981), red beetle (T. castaneum GenBank ID: EEZ98090.1), human body louse (P. humanus corporis GenBank ID: XP_002425564), diatom (P. tricornutum CCAP 1055/1 GenBank ID: XP_002176385.1), purple sea urchin (S. purpuratus GenBank ID: XM_001188537), water flea (D. pulex GenBank ID: EFX74110.1), fly (D. simulans protein GenBank ID: XP_002085048) and a second sea squirt gene (GenBank ID: XP_002122678.1). Global alignments of these putative proteins against human MDM2 and MDM4 orthologs showed sequence identities at or below 16 percent. Sequences with identities at or below 16 percent were excluded from further analysis because a sequence identity of 16 percent was achieved when shuffled and unshuffled human MDM2 sequences were aligned.

2.2 Amino acid ranges of protein domains

Table S2 (supplemental data) shows a list of protein domains used in this study and corresponding amino acid ranges used in this study. In the human species, amino acid ranges of domains within p63 and p73 that bind to MDM2 and MDM4 were obtained from sequence alignments with the p53 domain that binds to MDM2 and MDM4. Amino acid

[1]The term MDM is used to denote those MDM2 family members that cannot be clearly classified as MDM2 or MDM4.

sequences of domains from non-human orthologs were retrieved by multiple sequence alignment with the ClustalW2 software program (hosted by EMBL-EBI website) with default settings (Larkin et al., 2007).

2.3 Percent variation calculation and linear regression analysis

Ortholog domain sequences were individually aligned with human sequences with the Needleman-Wunsch EMBOSS Pairwise Alignment Algorithm hosted by the EMBL-EBI website (Needleman and Wunsch, 1970; Rice et al., 2000). Percent variation was calculated in these genes as the percent of non-identical amino acids observed in unsupervised global alignment with human homologs. Linear regression and correlation coefficients were determined with Microsoft Excel software. Estimates of divergence times for species were from the published sources. Chimpanzee, orangutan, macaque estimates are from Glazko and Nei, (Glazko and Nei, 2003). Rat, mouse, cat, dog, wild boar, elephant and hyrax are from Murphy et al., 2007 (Murphy et al., 2007). Owl limpet is from Putnam et al. 2008 (Putnam et al., 2008). All other estimates are from Dawkins, 2004 (Dawkins, 2004).

3.0 Results

3.1 MDM2 and MDM4 gene organization

When the Ensembl and GenBank databases were searched for *MDM2* gene sequences, 42 vertebrate species had partial length or full length sequences. Single *MDM2* orthologs were found in each species with the following exceptions: common marmoset (2 paralogs), rabbit (2 paralogs), tetraodon (2 paralogs). When these databases were searched for *MDM4* gene sequences, the same 42 species had one partial length or full length *MDM4* sequence. The vast majority of vertebrate organisms sequenced to date have one *MDM2* gene and one *MDM4* gene.

The human *MDM2* gene spans 11 exons and its coding sequence begins within the first exon and ends within the last exon (Table 1). The human *MDM4* gene spans 11 exons and its coding sequence begins within the second exon and ends within the last exon. The gene lengths of four *MDM2* genes and four *MDM4* genes were compared to determine if gene lengths were maintained across human, mouse, frog (X. tropicalis) and zebrafish species. The *MDM2* gene lengths in nucleotide units were 9,501 (zebrafish), 12,373 (frog), 21,871 (mouse) and 37,259 (human). The *MDM4* gene lengths were 25,578 (zebrafish), 149,800 (frog), 35,504 (mouse) and 41,738 (human). The significant sequence expansion in frog *MDM4* gene appears to be due to a relatively high number of Long Interspersed Nuclear Elements (LINES) located in introns 2, 3, 6, 7, and 9.

Notwithstanding the highly variable gene lengths, if *MDM2* and *MDM4* genes arose from a gene duplication event, one would expect coding exon lengths to be maintained in these species. Out of the 11 exons that constitute *MDM2* and *MDM4*, exons 3 through 10 fully code for protein in these four species. The nucleotide lengths of these coding exons have a standard deviation of 11 percent or less and an average percent standard deviation of 6 percent. Zebrafish is a bony fish, which shared a common ancestor with humans at least 440 million years ago within the Paleozoic era (Dawkins, 2004). Distinct *MDM2* and *MDM4* genes were not detected in invertebrates, although single MDM genes are detected (see section 3.5). The maintenance of coding exon lengths throughout approximately 440 million years of evolution within *MDM2* and *MDM4* indicates that the two *MDM2* family members arose through a gene duplication event prior to the appearance of bony vertebrates.

3.2 Rates of amino acid sequence variation within MDM2 and MDM4

Since their duplication more than 440 million years ago, both *MDM2* and *MDM4* have been subject to varying levels of selection pressure. We define percent variation in these genes as the percent of non-identical amino acids observed in unsupervised global alignment with human homologs (Needleman and Wunsch, 1970). Figure 1 shows percent variation in full-length MDM2 and MDM4 proteins as a function of the number of years of divergence from the last ancestor shared by humans and other species (Zuckerkandl and Pauling, 1962; Margoliash, 1963). The rate of evolution is similar for both genes, varying by only 2.3% assuming a linear rate. The correlation coefficient for MDM2 is 0.963 and the correlation coefficient for MDM4 is 0.942. Overall, MDM2 and MDM4 appear to be under a similar degree of selection pressure during all times since the emergence of bony vertebrates. This analysis does not take into consideration that subregions within MDM2 and MDM4 may have experienced different selection pressures (see section 3.4).

In the search for MDM2 and MDM4 protein homologs in more primitive organisms, seven invertebrate organisms were predicted to contain genes that code for homologs (see section 3.5). The invertebrate organisms contain only single MDM homologs. In Figure 1, the percent variation in the predicted MDM proteins of these invertebrate homologs was compared to human MDM2 and MDM4 (data points within rectangle section). The invertebrate MDM proteins are almost equally divergent from human MDM2 and MDM4.

3.3 Domain structure of MDM2 and MDM4 proteins

An understanding of the domain structure of the MDM2 protein family is necessary to analyze the evolution of the subregions. The longest transcript produced by the human *MDM2* gene codes for a protein that is 491 amino acids and the longest transcript produced by the human MDM4 gene codes for a protein 490 amino acids. The domain structures of the two proteins are almost identical, so MDM2 will be used as the primary example and differences with MDM4 will be highlighted later.

Four domains within human MDM2 are conserved in the jawed vertebrate species: the p53 binding domain, the acidic domain, the zinc finger, and the RING domain (Figure 2). These domains provide structure and/or functions that significantly contribute to MDM2's role as a regulator of p53. MDM2 residues 50–104 code for the p53 binding domain that forms a hydrophobic cleft into which the p53 α-helix binds (Kussie et al., 1996). The p53 α-helix is comprised of amino acids 18–26, which is part of a larger transactivation domain that binds to several transcription accessory factors.

MDM2 residues 243–301 comprise the acidic domain with a calculated pI of 2.9, owing to its large numbers of Asp and Glu amino acids. This domain contains four serines that are targets for phosphorylation by checkpoint kinase 1 and checkpoint kinase 2 (Weber et al., 1999). The acidic domain binds to the tumor suppressor, $p14/p19^{Arf}$, which leads to sequestration of MDM2 to the nucleolus. Sequestration of MDM2 precludes binding to p53, resulting in increased p53 activity and $p14/p19^{Arf}$ binding to MDM2 prevents MDM2-mediated ubiquitination of p53 (Hock and Vousden, 2010).

Downstream of the acidic domain is the zinc finger that encompasses amino acids 299–328. The zinc finger contains four conserved cysteines that coordinate zinc (Yu et al., 2006) and together, the zinc finger and acidic domain bind to several biological molecules including ribosomal proteins L5, L11, and L23, TATA binding protein, and Rb tumor suppressor.

The MDM2 RING domain, residues 438–479, is required for polyubiquitination of p53. The RING domain is also necessary for MDM2 homo-oligomerization and for hetero-dimerization with MDM4 (Kostic et al., 2006; Linke et al., 2008). The RING domain uses

six cysteines and two histidines to coordinate two zinc atoms. The MDM2 RING domain is required for transfer of ubiquitin from E2 onto Lys amino acids of p53 (Li et al., 2003). Polyubiquitination marks p53 for degradation by the 26S proteasome. The RING domain is also necessary for self destruction of MDM2, as it ubiquinates itself and its dimer partner MDM4. The RING domain of MDM4 does not possess E3 ligase activity. In addition to maintenance of the four conserved domains that MDM4 shares with MDM2, MDM4 binds to the N-terminal domain of p53 (Shvarts et al., 1996) and can also bind to and is sequestered into the nucleolus by $p14/p19^{Arf}$(Jackson et al., 2001).

3.4 Percent variation in domains of MDM2 and MDM4

The average rates at which MDM2 and MDM4 have accumulated substitutions are very similar for both genes, going back to the time of their duplication. Yet the rates are different for individual domains. Figure 2 shows the percent variation between human and other vertebrate species in the p53 binding domain, acidic domain, zinc finger, and RING domains.

As expected, percent variation in all domains increase as organisms are more distantly related to humans. The highest conservation was observed in the RING domain, and most variability observed in the acidic domain. Still, these rates are gene specific. If one considers the jawed bony fish species, stickleback, fugu, tetraodon, and zebrafish, the p53 binding domain of MDM2 accumulated changes faster than the analogous domain of MDM4. Similarly, the zinc finger of MDM2 varies more than the zinc finger of MDM4. On the other hand, percent variation in acidic domain and RING domain is greater in MDM4 than in MDM2. Several domains show increased conservation within placental animals. We note that increased conservation in the p53 family genes, p63 and p73 genes also was observed in placental animals (Belyi et al., 2010).

3.5 Invertebrate MDM family members

There are now seven invertebrate homologs of *MDM* genes. This includes previously published *MDM* genes in sea squirt (C. intestinalis), bay mussel (M. troussulus), Florida lancelet (B. floridae), owl limpet (L. giagantea), placozoans (T. adhaerens) and Northern deer tick (I. scapularis) (Lane et al., 2010a; Lane et al., 2010b; Muttray et al., 2010). Here, we report a seventh predicted invertebrate *MDM* from acorn worm (S. kowalevskii). Of the invertebrate MDM proteins, only bay mussel MDM was demonstrated to exist experimentally and shown to form a complex with p53 *in vitro* (Muttray et al., 2010). Although estimates vary, the most evolutionarily distant species of the invertebrates is placozoans which last shared a common ancestor with humans approximately 780 million years ago (Dawkins, 2004).

All seven invertebrate species appear to have only one MDM gene. The percent identity shared with human MDM2 ranged from 21 to 27 percent and the percent identity shared with human MDM4 ranged from 19 to 26 percent (Table 2). From this data, invertebrate MDMs appear to be equally divergent from MDM2 and MDM4. The most conserved domain within the invertebrates is the RING domain (Table 3). It is 32 to 57 percent different from the human MDM2 RING domain, and 52 to 59 percent different from human MDM4 RING domain. The MDM RING domains are more closely related to human MDM2 than to MDM4.

Sea squirt MDM protein was identified by automated computational analysis and transcripts of sea squirt MDM have been confirmed by EST and mRNA analysis. Putative owl limpet MDM was identified by Muttray's group (Muttray et al., 2010), which also identified bay

mussel MDM (also known as blue mussel). In bay mussel, MDM expression levels directly correlate with p53 family member expression in healthy and neoplastic hemocytes.

By comparative genomics, MDM homologs were reported in placozoans and deer tick (Lane et al., 2010a; Lane et al., 2010b). Placozoans are ocean floor dwelling multicellular organisms a few millimeters in diameter and have only three cell layers (Miller and Ball, 2005). The bottom layer contains cylinder cells which have cilia for locomotion and gland cells that secrete digestive enzymes. The top layer is composed of monociliated epithelial cells called cover cells. Between these two layers is a syncytium, a liquid-filled cavity with starlike struts. The placozoan genome is predicted to express over 11,000 genes, nearly 87 percent of which are homologs to known genes of other animals (Srivastava et al., 2008). Northern deer tick is the first species from the phylum Arthropoda predicted to code for MDM.

3.6 Acorn worm MDM

We show evidence for an MDM transcript in acorn worm. Using human MDM2 as a query, we performed Psi-BLAST (Altschul et al., 1997) analysis of non-vertebrate sequences in GenBank and discovered a transcript (GenBank ID: XP_002739076) from the acorn worm with significant identity to human MDM2 in its four conserved domains.2 Figure 3 is a schematic diagram that compares human MDM2 to predicted acorn worm MDM. The four conserved domains are maintained in the same order in the two species, but in acorn worm MDM there are added sequences at the amino terminus and between the zinc finger and RING domain. The putative acorn worm MDM is an 868 amino acid protein, which is 76 percent longer than human MDM2.

Analysis of the four conserved domains within the acorn worm sequence suggests that the protein is similar to MDM2. Within the p53 binding domain, acorn worm MDM shares 40 percent identity with human MDM2 and six out of 14 conserved hydrophobic and aromatic amino acids that are critical for p53 binding are identical. In the acidic domain the acorn worm MDM shares 37 percent identity with human MDM2. The acidic domain of human MDM2 has a calculated pI of 2.9 while acorn worm MDM has a calculated pI of 3.7. Within the zinc finger, human MDM2 and acorn worm MDM share 43 percent identity. Importantly, the four zinc-binding cysteines within human MDM2 are conserved in acorn worm MDM. Human MDM2 RING domain shares 57 percent identity with acorn worm MDM2 RING domain. The six cysteines and two histidines that bind to two zinc atoms in human MDM2 are conserved in acorn worm MDM. MDM has a phenylalanine in the penultimate position of the carboxyl terminus. The analogous position in mouse MDM2 is necessary for p53 ubiquitination and contributes to MDM2–MDM4 heterodimerization (Uldrijan et al., 2007). In both mouse and human MDM2 this position is occupied by tyrosine, but experimental replacement with phenylalanine preserves these critical functions.

The observation of a putative MDM homolog in acorn worm would suggest that this organism also codes for a p53/p63/p73 homolog. We identified three putative p53/p63/p73 paralogs in acorn worm (GenBank IDs: NP_001161624, XP_002732135, XP_002738810) two of which are full length. One full length sequence, NP_001161624, exhibits 30% identity with human p53, 28% identity with human p63 and 29% identity with human p73. The other full length sequence, XP_002732135, exhibits 10% identity with human p53, 24% identity with human p63, and 24% identity with human p73. One of the full length paralogs,

[2]This is considered significant identity because the identities of the four conserved domains shared between acorn worm MDM and human MDM2 are nearly the same as the identities shared between bay mussel MDM and human MDM2 conserved domains. The latter two proteins form a complex in vitro (Muttray et al., 2010). The identities between the four conserved domains in bay mussel MDM and human MDM2 are as follows: p53 binding domain, 38%; acidic domain, 28%; Zn finger, 43%; RING, 60%.

NP_001161624, shares an exon structure similar to that of the human *p53/p63/73* gene family. The other two paralogs are intronless and are likely the result of species-specific gene duplication, as all three genes exhibit 90% amino acid identity. We have found that all MDM-containing invertebrate species also code for p53/p63/p73 homologs, making it likely that MDM inhibits p53 family members in these organisms.

3.7 Comparison of the MDM2- and p53-family interaction domains

All three members of the p53 family, including p53, p63 and p73, code for MDM2 binding domains with the consensus sequence XFXXXWXXL. Structure studies show that Phe, Trp, and Leu side chains of human p53 are buried deep within a hydrophobic pocket of human MDM2 (Kussie et al., 1996) and human MDM4 (Popowicz et al., 2007; Kallen et al., 2009). Experimental evidence also shows that p73 binds to MDM2 (Mavinahalli et al., 2010). p53 and p73 bind to MDM4 through their MDM2 binding domains (Wang et al., 2001). One might expect the MDM2 binding domains of p53 to vary in a correlative fashion with the p53 binding domains of MDM2 and MDM4. Table 4 shows the percent variation in these interacting domains in vertebrate species. Within jawed vertebrates, the p53 binding domains within MDM2/4 vary from 0 to 28–38 percent, which approaches the percent variation in the MDM2 binding domain of p53 proteins (0–67 percent), while p73 orthologs show much higher conservation in their MDM2 binding domains (0–11 percent variation). The p63 orthologs have no variation throughout placental vertebrates, but then high percent variation within the bony fishes (56%). This relatively high conservation in p73 was surprising so we explored whether this reflected the conservation trend within the larger transactivation domains of these proteins.

The MDM2 binding domains within the p53 family members are located within larger domains called transactivation domains. The sequence conservation of the transactivation domains follows the pattern of MDM2 binding domain for p53 (0–81 percent variation) and p63 (0–76 percent variation), but not for p73 (0–51 percent variation). The pronounced difference between the percent variation in the MDM2 binding domains and the percent variation in the transactivation domains of p73 homologs may indicate a novel function of their MDM2 binding domains that is distinct from their MDM2 binding properties.

4.0 Discussion

4.1 *MDM2* and *MDM4* are the result of a duplication event more than 440 million years ago

Genetic and biochemistry data in mammals show that MDM2 and MDM4 proteins are critical for binding to p53 and inhibiting p53 transactivation activity. Both MDM2 family proteins also bind to and inhibit the p53 ortholog, p73. The evidence presented here suggests that more than 440 million years ago, a single *MDM* gene underwent duplication to form *MDM2* and *MDM4*. The fact that *MDM2* and *MDM4* in humans, mice, frogs and fish have very similar exon lengths (for those exons that fully code for protein) adds credence to the model that *MDM2* and *MDM4* are duplicated genes (see Table 1). Within vertebrate species for which there is complete genomic sequence available, there are, with rare exceptions, two *MDM* genes classified as *MDM2* and *MDM4*. We present evidence that only one *MDM* gene is coded in seven invertebrate species.

For invertebrate species, we find that invertebrates that code for *MDM* also code for the *p53/p63/p73* gene but the converse is not true. We failed to detect *MDM* in the monocellular eukaryote Choanoflagellate (M. brevicollis), D. melanogaster and C. elegans, for which there is reasonable evidence for the existence of *p53* family members (Jin et al., 2000; Derry et al., 2001; Nedelcu and Tan, 2007; King et al., 2008). It is likely that another p53 inhibitor can substitute for MDM2 protein as was found in C. elegans (Bergamaschi et al., 2003).

4.2 Average rates of evolution are similar for *MDM2* and *MDM4* but some differences are detected in conserved domains

To determine whether *MDM2* and *MDM4* are evolving at similar rates, the percent variation of their protein products in jawed vertebrates was calculated. Our data indicate that MDM2 and MDM4 proteins, on average, evolved at similar rates (see Figure 1) possibly due to similar selection pressure. This observation must be balanced against the fact that specific domains evolved at different rates.

MDM2 and MDM4 have four conserved domains defined by structure and function: the p53 binding domain, the acidic domain, the zinc finger, and the RING domain. The percent variation of these domains is not identical within MDM2 and MDM4 (see Figure 2). The acidic and RING domains are more conserved in MDM2 than in MDM4. The p53 binding domain and the zinc finger are generally more conserved in MDM4 than in MDM2. This is not entirely surprising since these domains likely have features that cause MDM2 and MDM4 to have some distinct functions. These functions could affect p53 inhibition in different ways, to different degrees, or even be p53-independent.

Within the jawed vertebrate MDM2 and MDM4 proteins the most conserved domain is the MDM2 RING. Within MDM2 proteins the percent variation range is 0–21 percent, almost twice lower than the 0–43 percent variation range in MDM4. The MDM2 RING is required for heterodimerization with MDM4 (through MDM4's RING) and homo-oligomerization. Two features distinguish the MDM2 RING and the MDM4 RING. First, the MDM2 RING is an E3 ubiquitin ligase and MDM4 RING is not. Second, MDM2 can form homo-oligomers through its RING but MDM4 does not (Tanimura et al., 1999). Thus, the MDM2 and MDM4 RING domains possess measurable biochemical differences that are reflected by their highly different levels of percent variation.

The MDM2 RING finger contains a conserved cysteine at position 447 in vertebrates. When Cys447 is mutated MDM2 it loses E3 ubiquitin ligase activity but retains MDM4 heterodimerization properties (Fang et al., 2000; Singh et al., 2007). None of the invertebrate species MDMs have Cys447, which means that they are unlikely to have E3 activity. MDM2 mutants that fail to heterodimerize with MDM4 are Ile448Glu and Ile483Glu (Singh et al., 2007). Invertebrate MDMs either retain Ile at these positions or have very conservative amino acid substitutions. MDM4 and MDM2 compete for identical binding sites on the MDM2 RING finger. These observations support a model where oligomerization is an ancient property in MDM. Near the time of the duplication event the RING finger acquired E3 ubiquitin ligase activity. Once this activity was achieved, relatively little percent variation in MDM2 RING was tolerated. The MDM4 protein lost its ability to homo-oligomerize but retained its ability to heterodimerize.

4.3 Putative invertebrate MDM proteins have conserved RING domains

In seven invertebrate species, MDM proteins shared 19–27 percent identities with human MDM2 and MDM4 (see Table 2). The most distant homolog is found in placozoans, which last shared a common ancestor with humans approximately 780 million years ago (Dawkins, 2004; Lane et al., 2010a). Consistently, the invertebrate MDM domain most identical to human MDM2 and MDM4 is the RING domain (see Table 3). The RING domain (also known as the RING-type Zn finger) is a highly conserved zinc-binding motif (see accession number IPR001841 on the InterPro website) found in multiple invertebrates including seven examples from archaea (Hunter et al., 2009).

4.4 The p53 binding domain of MDM2 and MDM4 coevolved with its interaction partner within p53

The general trend in percent variation n the p53 binding domain of MDM2 and the MDM2 binding domain of p53 (see Table 4) suggests that these interacting domains may be undergoing coadaptation, defined as a change in one gene family that will influence changes in another gene family (Pazos and Valencia, 2008). In this case, coadaptation requires physical interaction between the *MDM2* gene family products and the *p53* gene family products. The matching trend is also observed in the interacting domains of p53 and MDM4. Surprisingly, the sequence of the MDM2 binding domain of p73 remains nearly constant throughout the jawed vertebrates, unlike p53. This nearly constant binding domain lies within a larger transactivation domain that exhibits high percent variation. The strong conservation of the binding domain is not attributable to the entire transactivation domain of p73 but, rather, just the short 9 amino acid sequence that binds to MDM2. This suggests that there may be an MDM2/4-independent function for the MDM2 binding domain of p73.

4.5 Conclusions

The *MDM2* gene family is composed of *MDM2* and *MDM4* which resulted from a duplication event prior to the appearance of bony vertebrates more than 440 million years ago. The most conserved domain in the MDM2 protein is the RING domain. Seven invertebrate species are predicted to express MDM proteins, the most primitive of which is placozoans, an organism that shared a common ancestor with humans approximately 780 million years ago. The domain within invertebrate MDMs that is most identical to human MDM2 is the RING domain.

We propose that ancient *p53/p63/p73* gene first evolved in the ancestor common to placozoans and choanoflagellates where it functioned to protect germline cells from DNA damage. Emerging evidence suggests that placozoans have the machinery for sexual reproduction (Eitel et al., 2011). Choanoflagellates are single celled and form colonies, but it is unclear whether they can undergo sexual reproduction. Existing data shows that MDM first appeared in placozoans. *MDM* was not detected in sponge (A. queenslandica), choanoflagellates, plants, bacteria, or archaea. In choanoflagellates (as well as some other invertebrates) there are probably non-*MDM* inhibitors of *p53/p63/p73*. More than 440 million years ago with the emergence of bony vertebrates, three distinct *p53* family genes were selected for: *p53*, *p63*, and *p73*. This is also the time period where *MDM* was duplicated and *MDM2* and *MDM4* became the primary negative regulators of *p53*. Near the time of duplication MDM2 is likely to have acquired E3 ubiquitin ligase activity while MDM4 retained the more ancient MDM function of limited oligomerization in order to heterodimerize with MDM2.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grant number 1 P20 CA118783 to JM. VB was supported by Martin A. and Helen Chooljian membership at the Institute for Advanced Study. The authors wish to thank Prashanth Ak and Asad Naqvi for helpful discussions, Wayne Warner for critical reading of the manuscript, and Scott Grover for use of the SigmaPlot software program. JM thanks Arnold Levine for the opportunity to begin this research at the Institute for Advanced Study.

References

Altschul SF, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997; 25:3389–3402. [PubMed: 9254694]

Araki S, et al. TGF-beta1-induced expression of human Mdm2 correlates with late-stage metastatic breast cancer. J Clin Invest. 2010; 120:290–302. [PubMed: 19955655]

Balint E, Reisman D. Increased rate of transcription contributes to elevated expression of the mutant p53 gene in Burkitt's lymphoma cells. Cancer Res. 1996; 56:1648–1653. [PubMed: 8603415]

Belyi VA, et al. The origins and evolution of the p53 family of genes. Cold Spring Harb Perspect Biol. 2010; 2 a001198.

Bergamaschi D, et al. iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human. Nat Genet. 2003; 33:162–167. [PubMed: 12524540]

Bond GL, et al. MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. Cancer Res. 2006; 66:5104–5110. [PubMed: 16707433]

Brandt T, Petrovich M, Joerger AC, Veprintsev DB. Conservation of DNA-binding specificity and oligomerisation properties within the p53 family. BMC Genomics. 2009; 10:628. [PubMed: 20030809]

Cahilly-Snyder L, Yang-Feng T, Francke U, George DL. Molecular analysis and chromosomal mapping of amplified genes isolated from a transformed mouse 3T3 cell line. Somat Cell Mol Genet. 1987; 13:235–244. [PubMed: 3474784]

Calabro V, et al. The human MDM2 oncoprotein increases the transcriptional activity and the protein level of the p53 homolog p63. J Biol Chem. 2002; 277:2674–2681. [PubMed: 11714701]

Candau R, et al. Two tandem and independent sub-activation domains in the amino terminus of p53 require the adaptor complex for activity. Oncogene. 1997; 15:807–816. [PubMed: 9266967]

Consortium TU. Ongoing and future developments at the Universal Protein Resource. Nucleic Acids Res. 2011; 39:D214–D219. [PubMed: 21051339]

Dawkins, R. The Ancestor's Tale: A Pilgrimage to the Dawn of Evolution. Houghton Mifflin, New York: 2004.

Derry WB, Putzke AP, Rothman JH. Caenorhabditis elegans p53: role in apoptosis, meiosis, and stress resistance. Science. 2001; 294:591–595. [PubMed: 11557844]

Eitel M, Guidi L, Hadrys H, Balsamo M, Schierwater B. New insights into placozoan sexual reproduction and development. PLoS One. 2011; 6:e19639. [PubMed: 21625556]

el-Deiry WS, et al. WAF1, a potential mediator of p53 tumor suppression. Cell. 1993; 75:817–825. [PubMed: 8242752]

Fakharzadeh SS, Trusko SP, George DL. Tumorigenic potential associated with enhanced expression of a gene that is amplified in a mouse tumor cell line. Embo J. 1991; 10:1565–1569. [PubMed: 2026149]

Fang S, Jensen JP, Ludwig RL, Vousden KH, Weissman AM. Mdm2 is a RING finger-dependent ubiquitin protein ligase for itself and p53. J Biol Chem. 2000; 275:8945–8951. [PubMed: 10722742]

Flicek P, et al. Ensembl 2011. Nucleic Acids Res. 2011; 39:D800–D806. [PubMed: 21045057]

Glazko GV, Nei M. Estimation of divergence times for major lineages of primate species. Mol Biol Evol. 2003; 20:424–434. [PubMed: 12644563]

Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. Nature. 1997; 387:296–299. [PubMed: 9153395]

Hock A, Vousden KH. Regulation of the p53 pathway by ubiquitin and related proteins. Int J Biochem Cell Biol. 2010; 42:1618–1621. [PubMed: 20601087]

Honda R, Tanaka H, Yasuda H. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. FEBS Lett. 1997; 420:25–27. [PubMed: 9450543]

Hubbard TJ, et al. Ensembl 2009. Nucleic Acids Res. 2009; 37:D690–D697. [PubMed: 19033362]

Hunter S, et al. InterPro: the integrative protein signature database. Nucleic Acids Res. 2009; 37:D211–D215. [PubMed: 18940856]

Jackson MW, Lindstrom MS, Berberich SJ. MdmX binding to ARF affects Mdm2 protein stability and p53 transactivation. J Biol Chem. 2001; 276:25336–25341. [PubMed: 11297540]

Jin S, et al. Identification and characterization of a p53 homologue in Drosophila melanogaster. Proc Natl Acad Sci U S A. 2000; 97:7301–7306. [PubMed: 10860994]

Kadakia M, Slader C, Berberich SJ. Regulation of p63 function by Mdm2 and MdmX. DNA Cell Biol. 2001; 20:321–330. [PubMed: 11445003]

Kallen J, et al. Crystal Structures of Human MdmX (HdmX) in Complex with p53 Peptide Analogues Reveal Surprising Conformational Changes. J Biol Chem. 2009; 284:8812–8821. [PubMed: 19153082]

King N, et al. The genome of the choanoflagellate Monosiga brevicollis and the origin of metazoans. Nature. 2008; 451:783–788. [PubMed: 18273011]

Kojima T, Ikawa Y, Katoh I. Analysis of molecular interactions of the p53-family p51(p63) gene products in a yeast two-hybrid system: homotypic and heterotypic interactions and association with p53-regulatory factors. Biochem Biophys Res Commun. 2001; 281:1170–1175. [PubMed: 11243857]

Kostic M, Matt T, Martinez-Yamout MA, Dyson HJ, Wright PE. Solution structure of the Hdm2 C2H2C4 RING, a domain critical for ubiquitination of p53. J Mol Biol. 2006; 363:433–450. [PubMed: 16965791]

Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. Nature. 1997; 387:299–303. [PubMed: 9153396]

Kussie PH, et al. Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. Science. 1996; 274:948–953. [PubMed: 8875929]

Lane DP, et al. Mdm2 and p53 are highly conserved from placozoans to man. Cell Cycle. 2010a; 9:540–547. [PubMed: 20081368]

Lane DP, et al. The Mdm2 and p53 genes are conserved in the Arachnids. Cell Cycle. 2010b; 9:748–754. [PubMed: 20160485]

Larkin MA, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007; 23:2947–2948. [PubMed: 17846036]

Laurie NA, et al. Inactivation of the p53 pathway in retinoblastoma. Nature. 2006; 444:61–66. [PubMed: 17080083]

Li M, et al. Mono- versus polyubiquitination: differential control of p53 fate by Mdm2. Science. 2003; 302:1972–1975. [PubMed: 14671306]

Liang M, et al. HDM4 is overexpressed in mantle cell lymphoma and its inhibition induces p21 expression and apoptosis. Mod Pathol. 2010; 23:381–391. [PubMed: 20062013]

Linares LK, Hengstermann A, Ciechanover A, Muller S, Scheffner M. HdmX stimulates Hdm2-mediated ubiquitination and degradation of p53. Proc Natl Acad Sci U S A. 2003; 100:12009–12014. [PubMed: 14507994]

Linke K, et al. Structure of the MDM2/MDMX RING domain heterodimer reveals dimerization is required for their ubiquitylation in trans. Cell Death Differ. 2008; 15:841–848. [PubMed: 18219319]

Little NA, Jochemsen AG. Hdmx and Mdm2 can repress transcription activation by p53 but not by p63. Oncogene. 2001; 20:4576–4580. [PubMed: 11494153]

Margoliash E. Primary Structure and Evolution of Cytochrome C. Proc Natl Acad Sci U S A. 1963; 50:672–679. [PubMed: 14077496]

Mavinahalli JN, Madhumalar A, Beuerman RW, Lane DP, Verma C. Differences in the transactivation domains of p53 family members: a computational study. BMC Genomics. 2010; 11 Suppl 1:S5. [PubMed: 20158876]

Miller DJ, Ball EE. Animal evolution: the enigmatic phylum placozoa revisited. Curr Biol. 2005; 15:R26–R28. [PubMed: 15649348]

Miller W, et al. 28-way vertebrate alignment and conservation track in the UCSC Genome Browser. Genome Res. 2007; 17:1797–1808. [PubMed: 17984227]

Momand J, Jung D, Wilczynski S, Niland J. The MDM2 gene amplification database. Nucleic Acids Res. 1998; 26:3453–3459. [PubMed: 9671804]

Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell. 1992; 69:1237–1245. [PubMed: 1535557]

Murphy WJ, Pringle TH, Crider TA, Springer MS, Miller W. Using genomic data to unravel the root of the placental mammal phylogeny. Genome Res. 2007; 17:413–421. [PubMed: 17322288]

Muttray AF, O'Toole TF, Morrill W, Van Beneden RJ, Baldwin SA. An invertebrate mdm homolog interacts with p53 and is differentially expressed together with p53 and ras in neoplastic Mytilus trossulus haemocytes. Comp Biochem Physiol B Biochem Mol Biol. 2010; 156:298–308. [PubMed: 20417299]

Nedelcu AM, Tan C. Early diversification and complex evolutionary history of the p53 tumor suppressor gene family. Dev Genes Evol. 2007; 217:801–806. [PubMed: 17924139]

Needleman SB, Wunsch CD. A General Method Applicable to the Search for Similarities in Amino Acid Sequence of Two Proteins. J. Mol. Biol. 1970; 48:443–453. [PubMed: 5420325]

Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B. Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature. 1992; 358:80–83. [PubMed: 1614537]

Pazos F, Valencia A. Protein co-evolution, co-adaptation and interactions. Embo J. 2008; 27:2648–2655. [PubMed: 18818697]

Popowicz GM, et al. Molecular basis for the inhibition of p53 by Mdmx. Cell Cycle. 2007; 6:2386–2392. [PubMed: 17938582]

Prasad AB, Allard MW, Green ED. Confirming the phylogeny of mammals by use of large comparative sequence data sets. Mol Biol Evol. 2008; 25:1795–1808. [PubMed: 18453548]

Putnam NH, et al. The amphioxus genome and the evolution of the chordate karyotype. Nature. 2008; 453:1064–1071. [PubMed: 18563158]

Rice P, Longden I, Bleasby A. EMBOSS: the European Molecular Biology Open Software Suite. Trends Genet. 2000; 16:276–277. [PubMed: 10827456]

Rutkowski R, Hofmann K, Gartner A. Phylogeny and function of the invertebrate p53 superfamily. Cold Spring Harb Perspect Biol. 2010; 2 a001131.

Scoumanne A, Harms KL, Chen X. Structural basis for gene activation by p53 family members. Cancer Biol Ther. 2005; 4:1178–1185. [PubMed: 16294021]

Shvarts A, et al. MDMX: a novel p53-binding protein with some functional properties of MDM2. Embo J. 1996; 15:5349–5357. [PubMed: 8895579]

Singh RK, Iyappan S, Scheffner M. Hetero-oligomerization with MdmX rescues the ubiquitin/Nedd8 ligase activity of RING finger mutants of Mdm2. J Biol Chem. 2007; 282:10901–10907. [PubMed: 17301054]

Srivastava M, et al. The Trichoplax genome and the nature of placozoans. Nature. 2008; 454:955–960. [PubMed: 18719581]

Tanimura S, et al. MDM2 interacts with MDMX through their RING finger domains. FEBS Lett. 1999; 447:5–9. [PubMed: 10218570]

Toledo F, Wahl GM. Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. Nat Rev Cancer. 2006; 6:909–923. [PubMed: 17128209]

Uldrijan S, Pannekoek WJ, Vousden KH. An essential function of the extreme C-terminus of MDM2 can be provided by MDMX. Embo J. 2007; 26:102–112. [PubMed: 17159902]

Wade M, Wang YV, Wahl GM. The p53 orchestra: Mdm2 and Mdmx set the tone. Trends Cell Biol. 2010; 20:299–309. [PubMed: 20172729]

Wang X, et al. MDM2 and MDMX can interact differently with ARF and members of the p53 family. FEBS Lett. 2001; 490:202–208. [PubMed: 11223036]

Weber JD, Taylor LJ, Roussel MF, Sherr CJ, Bar-Sagi D. Nucleolar Arf sequesters Mdm2 and activates p53. Nat Cell Biol. 1999; 1:20–26. [PubMed: 10559859]

Yu GW, Allen MD, Andreeva A, Fersht AR, Bycroft M. Solution structure of the C4 zinc finger domain of HDM2. Protein Sci. 2006; 15:384–389. [PubMed: 16385008]

Zdzalik M, et al. Interaction of regulators Mdm2 and Mdmx with transcription factors p53, p63 and p73. Cell Cycle. 2010; 9:4584–4591. [PubMed: 21088494]

Zeng X, et al. MDM2 suppresses p73 function without promoting p73 degradation. Mol Cell Biol. 1999; 19:3257–3266. [PubMed: 10207051]

Zuckerkandl, E.; Pauling, LB. Molecular disease, evolution, and genetic heterogeneity. In: Kasha, M.; Pullman, B., editors. Horizons in Biochemistry. New York: Academic Press; 1962. p. 189-225.

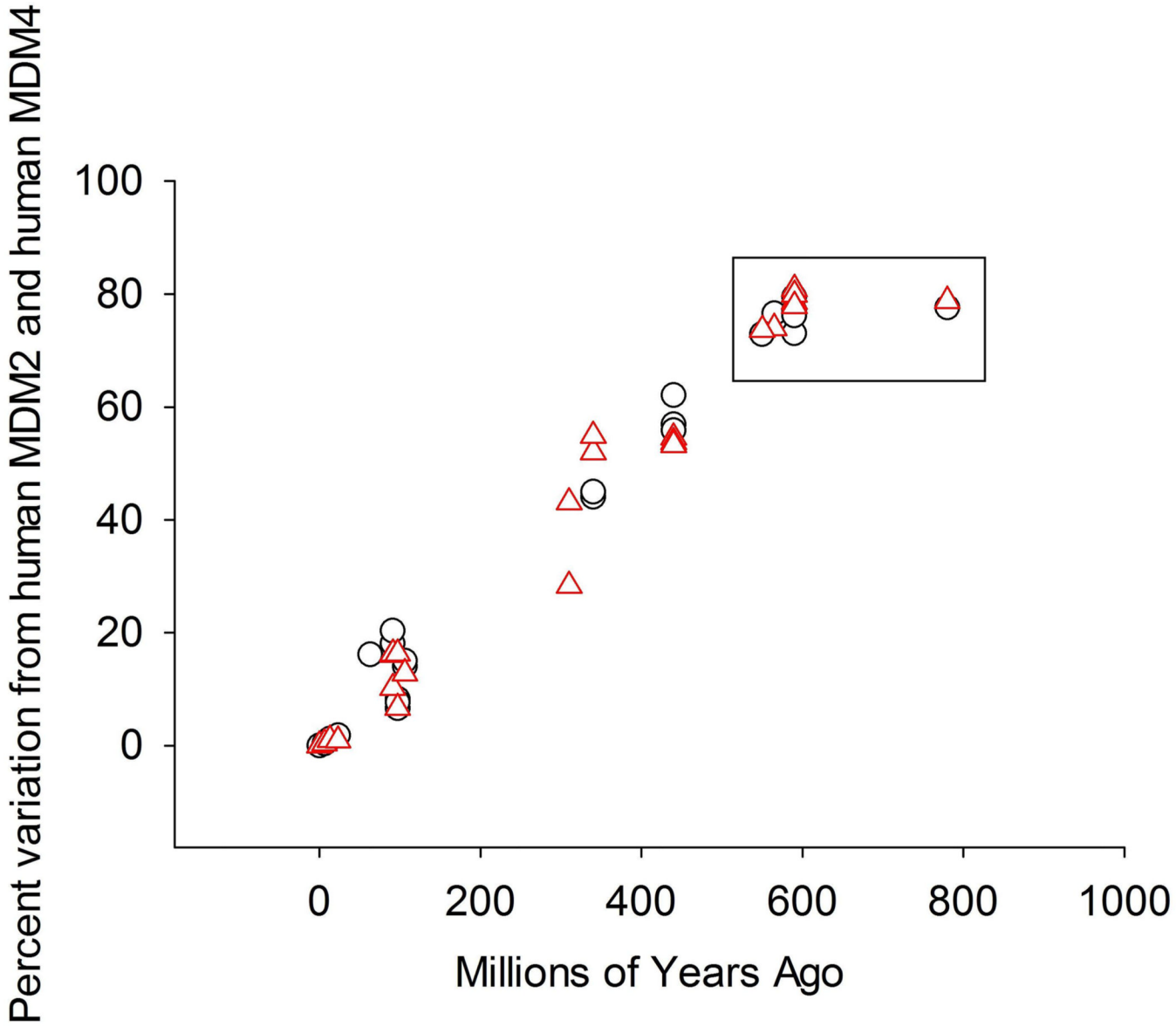
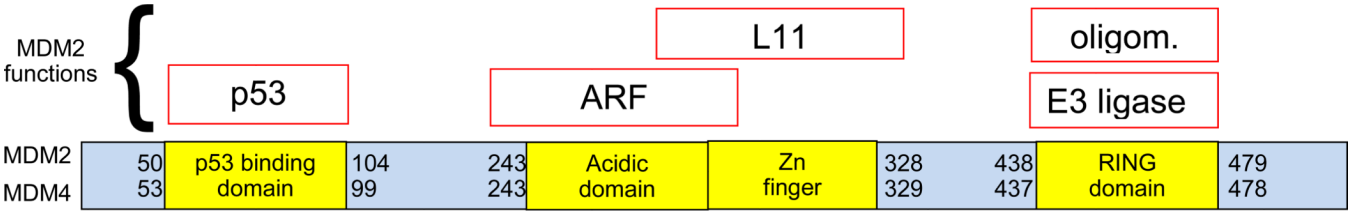


Figure 1.

Percent variation in the MDM2 and MDM4 protein sequences relative to their human orthologs. Percent variation of vertebrate MDM2 orthologs (black circles) and vertebrate MDM4 orthologs (red circles) is plotted versus time to the last known common ancestor. Percent variation in invertebrate MDM sequences, shown inside rectangular region, were calculated against both human MDM2 and MDM4 proteins. Only full length protein sequences were used in this plot.



	% variation			% variation		% variation			% variation	
	MDM2	MDM4		MDM2	MDM4	MDM2	MDM4		MDM2	MDM4
Human	0	0	----------	0	0	0	0	----------	0	0
Chimp	0	0	----------	2	2	0	0	----------	0	0
Orangutan	0	0	----------	3	2	0	3	----------	0	0
Macaque	0	0	----------	3	5	0	0	----------	0	0
Bushbaby	4	-	----------	9	8	20	0	----------	2	-
Cat	2	-	----------	2	-	3	-	----------	0	-
Dog	2	2	----------	3	5	3	0	----------	0	0
Wild boar	8	0	----------	3	-	3	3	----------	0	0
Elephant	9	6	----------	7	11	7	3	----------	2	2
Mouse	9	4	----------	8	18	7	0	----------	2	11
Rat	9	6	----------	8	20	3	0	----------	2	11
Hyrax	9	-	----------	10	26	0	0	----------	2	9
Chicken	18	17	----------	20	52	20	13	----------	7	36
Lizard	17	25	----------	25	47	27	17	----------	-	33
Frog (X. trop)	27	26	----------	41	57	37	23	----------	7	38
Frog (X. laev)	27	26	----------	39	57	37	20	----------	7	43
Stickleback	33	28	----------	42	56	40	30	----------	19	33
Zebrafish	38	28	----------	52	66	43	30	----------	12	33
Tetraodon	36	28	----------	47	62	37	33	----------	21	33
Fugu	33	28	----------	46	55	40	33	----------	21	31
Medaka	36	28	----------	42	65	40	30	----------	19	33

Figure 2.

Domain architecture of MDM2 family. Some MDM2 binding proteins and functional domains are depicted above the MDM2/4 protein schematic. The MDM2 binding proteins are p53 (p53), p14/p19^{arf} (ARF), L11 ribosomal protein (L11). The functional domains are the oligomerization domain (oligom.) and E3 ligase activity (E3 ligase). Below the binding proteins and functional domains is the MDM2/4 protein schematic. The top set of numbers corresponds to the amino acids that bind to conserved domains of MDM2. The bottom set of numbers corresponds to the amino acids that bind to conserved domains of MDM4. Below the MDM2/4 protein schematic is a table of percent variation in the conserved domains. Each value corresponds to the percent amino acid difference between given species and human MDM2 and human MDM4 domains. Only domains with complete sequences were given values. Wherever possible, species are listed in order of increasing evolutionary distance from humans (Miller et al., 2007; Prasad et al., 2008).

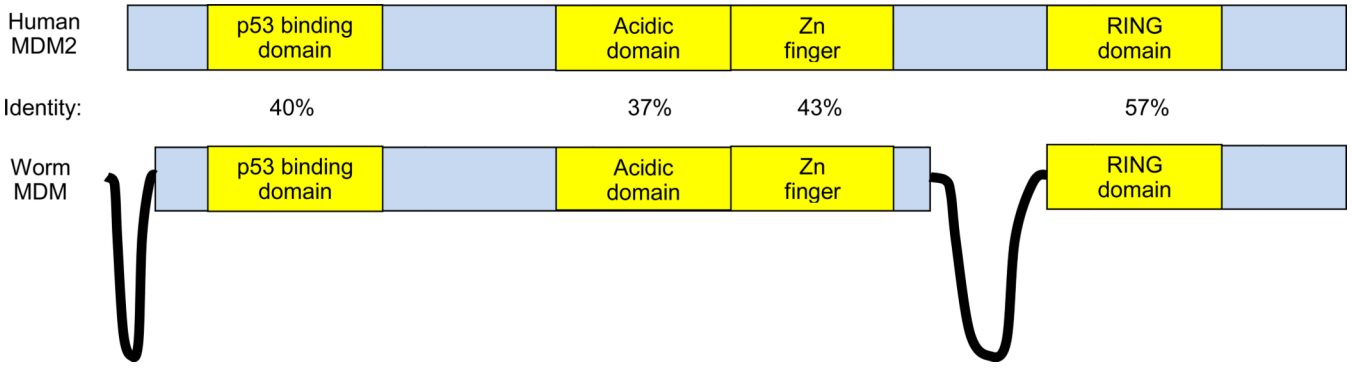


Figure 3.

Domain architecture of human MDM2 and putative acorn worm MDM. Percent identities were calculated for the conserved domains listed. Black loops are amino acid sequences within acorn worm MDM that are not similar to human MDM2.

Table 1

Exon structure of *MDM2* gene family members.[a]

	human MDM2		human MDM4		MDM2 and MDM4 homologs
full gene length	**37,259**		**41,738**		
exons	**protein coding**	**length**	**protein coding**	**length**	**ave. exon lengths**
Exon1	Partial	316	No	127	
Exon2	Full	85	Partial	113	89 ± 13
Exon3	Full	75	Full	75	75 ± 1
Exon4	Full	134	Full	134	134 ± 0
Exon5	Full	50	Full	56	52 ± 3
Exon6	Full	68	Full	68	64 ± 4
Exon7	Full	97	Full	100	95 ± 10
Exon8	Full	161	Full	161	161 ± 5
Exon9	Full	156	Full	150	158 ± 13
Exon10	Full	78	Full	81	79 ± 8
Exon11	Partial	6,161	Partial	9,008	3,132 ± 2,725

[a]Exons and coding regions for the longest transcripts were obtained from the Ensembl database, release 58 (Hubbard et al., 2009). Average exon lengths ± standard deviations were calculated from four MDM2 and four MDM4 genes from human, mouse, frog (X. tropicalis) and zebrafish (D. rerio). Exon 1 was not present in frog MDM4.

Table 2

Protein sequence identities of invertebrate MDMs shared with human MDM2 and human MDM4.

invertebrate species	percent identity to human MDM2	percent identity to human MDM4	MDM identification reference
Florida lancelet (B. floridae)	27	26	(Muttray et al., 2010)
Bay mussel (M. trossulus)	27	21	(Muttray et al., 2010)
Owl limpet (L. gigantean)	24	22	(Muttray et al., 2010)
Sea squirt (C. intestinalis)	23	26	Ensembl database
Deer tick (I. scapularis)	23	20	(Lane et al., 2010b)
Placozoans (T. adhaerens)	22	21	(Lane et al., 2010a)
Acorn worm (S. kowalevskii)	21	19	this study

Table 3

Percent variation in invertebrate MDM RING domains in comparison to human MDM2 RING domain and in comparison to human MDM4 RING domain.

invertebrate species	percent variation in human MDM2 RING	percent variation in human MDM4 RING
Lancelet	32	52
Owl limpet	40	50
Bay mussel	40	52
Acorn worm	43	52
Sea squirt	47	52
Deer tick	55	57
Placozoans	57	59

Table 4

Percent variation in the interaction domains of MDM2 and p53 protein families and percent variation in p53 protein family transactivation domains.[a]

	p53 BD in MDM2	p53 BD in MDM4	MDM2 BD in p53	MDM2 BD in p63	MDM2 BD in p73	p53 TD domain	p63 TD domain	p73 TD domain
Human	0	0	0	0	0	0	0	0
Chimp	0	0	0	0	0	0	0	0
Orangutan	0	0	0	0	0	3	0	0
Macaque	0	0	0	0	0	5	0	2
Bushbaby	4		0	0		25	7	
Cat	2		22		11	33		30
Dog	2	0	22		0	35		26
Elephant	9	6	22	0		25	7	
Mouse	9	4	11	0	0	48	10	22
Rat	9	6	11	0	0	44		23
Hyrax	9		22		11	35	2	
Frog (X. trop)	27		22		11	57		37
Frog (X. laev)	27	26	22			66		
Stickleback	33	26	67		0	75		48
Zebrafish	38	28	67		11	74		50
Tetraodon	36	28	44	56		81	72	51
Fugu	33	28	44	56	0	75	67	48
Medaka	36	28	67	56	0	73	76	

[a] For p53 binding domain (BD) in MDM2, each value corresponds to the percent variation between given species and human p53 binding domains within MDM2. BD in MDM4, each value corresponds to the percent variation between given species and human p53 binding domains within MDM4. For MDM2 BD in p53, p63, and p73 columns, each value corresponds to the percent variation between given species and human MDM2 binding domains within p53, p63 and p73. TD is the transactivation domain.