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The Comparative Proteomics of Ubiquitination in Mouse

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Ubiquitination is a common posttranslational modification in eukaryotic cells, influencing many fundamental cellular processes. Defects in ubiquitination and the processes it mediates are involved in many human disease states. The ubiquitination of a substrate involves four classes of enzymes: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), a ubiquitin protein ligase (E3), and a de-ubiquitinating enzyme (DUB). A substantial number of E1s (four), E2s (13), E3s (97), and DUBs (six) that were previously unknown in the mouse are included in the FANTOM2 Representative Transcript and Protein Set (RTPS). Many of the genes encoding these proteins will constitute promising candidates for involvement in disease. In addition, the RTPS provides the basis for the most comprehensive survey of ubiquitination-associated proteins across eukaryotes undertaken to date. Comparisons of these proteins across human and other organisms suggest that eukaryotic evolution has been associated with an increase in the number and diversity of E3s (possessing either zinc-finger RING, F-box, or HECT domains) and DUBs (containing the ubiquitin thiolesterase family 2 domain). These increases in numbers are too large to be accounted for by the presence of fragmentary proteins in the data sets examined. Much of this innovation appears to have been associated with the emergence of multicellular organisms, and subsequently of vertebrates, increasing the opportunity for complex regulation of ubiquitination-mediated cellular and developmental processes.

Ubiquitination is a central process in eukaryotic cells, influencing meiosis, cellular proliferation, and development. It is now seen as the most common posttranslational modification in all eukaryotes after phosphorylation. Until recently, it was thought that ubiquitin was simply a cellular label to target proteins for destruction in the proteasome, but it is now known to have additional roles in trafficking, kinase activation, and regulating gene expression (Conaway et al. 2002). These different roles are associated with different multi-ubiquitin chains, differing according to the particular lysine residue, within the ubiquitin molecules, which is used to interlink the chain (Weissman 2001). It appears that these different multi-ubiquitin chains, together with mono-ubiquitination, act as signals in different cell processes. Defects in ubiquitination are known to be involved in many human disease states such as developmental abnormalities, autoimmunity, neurodegenerative diseases (Alzheimer’s disease, Parkinson’s disease, Down’s syndrome, and normal aging of the brain), and cancer (Weissman 2001). Ubiquitination is a multistep process requiring four classes of enzyme: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), a ubiquitin protein ligase (E3), and a de-ubiquitinating enzyme (DUB). Each of these four classes contains characteristic domains represented in the InterPro domain database (Apweiler et al. 2000).

Ubiquitination begins when the E1 recruits ubiquitin in an ATP-dependent process; then the E2 accepts ubiquitin from the E1. The E3 catalyzes the transfer of ubiquitin from the E2 to the substrate. At the proteasome, DUBs cleave multi-ubiquitin chains from substrates and disassemble the chains. This ensures that ubiquitinated proteins remain associated with the proteasome and prevents the accumulation of residual multi-ubiquitin chains that can disrupt proteasome activity. DUBs also process immature ubiquitin, which is translated as a fusion protein that is either a chain of ubiquitin molecules or joined to small ribosomal subunits (Weissman 2001). The flexibility and complexity of cellular regulation by ubiquitination can be increased at each stage by increasing the numbers of proteins in each of the four classes. For example, many E3s are SCF (Skp1-Cullin-F-box protein) complexes composed of proteins containing four InterPro domains: SKP1 component (IPR001232), F-box (IPR001810), Cullin (IPR001373), and zinc-finger RING (IPR001841; Jackson and Eldridge 2002). The large numbers of proteins containing these domains mean that a huge number of different E3 complexes, recognizing a wide range of protein substrates, can be formed.

The Representative Transcript and Protein Set (RTPS) amalgamates cDNA clones produced in the recent RIKEN FANTOM2 project with other publicly available cDNA sequences to provide the first overview of the mouse transcriptome (The FANTOM Consortium and the RIKEN GER Group Phase I and II Team 2002). Previous comparisons of ubiquitination-associated (UA) proteins between species such as human, Caenorhabditis elegans, and yeast have suggested that the numbers and compositions of E2 and E3 proteins are related to developmental complexity (Von Arnim 2001; Jones et al. 2002). The RTPS provides valuable additional data to investigate such assertions and also to seek putatively vertebrate specific features of ubiquitination. In the present study, we de-
scribe the numbers and architectures of proteins containing UA InterPro domains across the known mouse proteome, in the light of the RTPS. These data are compared with the equivalent data from human, Drosophila melanogaster, C. elegans, Schizosaccharomyces pombe, and Saccharomyces cerevisiae proteomes. This study represents the most comprehensive survey of UA proteins across eukaryotes undertaken to date.

RESULTS

Table 1 shows the numbers of UA proteins present in three incomplete versions of the mouse proteome: the RTPS, a version derived from the SWISS-PROT and TrEMBL databases (SPTR), and a version derived from SWISS-PROT, TrEMBL, and the mouse Ensembl database (SPTRENS). It shows that the number of UA proteins within the RTPS exceeds the number within SPTR and constitutes more than three fourths of the number within SPTRENS. This suggests that the RTPS provides substantial coverage of the mouse transcriptome, because the SPTRENS includes proteins predicted from a high-quality mouse genome assembly produced by the Mouse Genome Sequencing Consortium (which has around seven times the coverage and covers an estimated 96% of the mouse euchromatic genome; see http://www.ensembl.org/Mus_musculus/). Of the 496 proteins present in the RTPS, 146 (30%) were found to be absent from the SPTRENS set (i.e., they lacked a matching sequence of ≥98% identity over ≥20 residues). Significant numbers of novel mouse proteins belonging to each enzyme class were discovered, though many have close human homologs.

The novel UA proteins from the RTPS were combined with those from the SPTRENS to provide the most complete set of mouse UA proteins possible; this combined set was then compared with sets of UA proteins from five other "draft" or completely sequenced organisms. Table 2 shows that higher numbers of E1 and E2 proteins are found in mouse and human than in the other organisms examined. This is also true of E3 proteins if one ignores the very high number of F-box (IPR001810) proteins in C. elegans (which remains mysterious; Kipreos and Pagano 2000), and the same observations can also be made of the remaining two classes (DUB and non-applicable [N/A] proteins). The differences and similarities between these organisms are often clearer at the level of the domains that define the enzyme classes. Within E3 proteins, the largest differences between organisms are seen in zinc-finger RING (IPR001841) or HECT (IPR000569) domains in particular, whereas within DUBs, the ubiquitin thiolesterase family 2 (IPR001394) domain shows the largest differences.

Within the N/A class, the largest differences are seen for the ubiquitin interacting motif (UIM; IPR003903) and the UBA domain (IPR000127) and constitute more than three fourths of the number of UA proteins within the RTPS exceeds the number in the other four classes. Table 3 shows the numbers of additional, non-UA proteins in UA proteins from each class. From Table 3, it is possible to see the ratio of the numbers of non-UA domains to the number of proteins they are present within. Usually, this ratio is ~1, reflecting little redundancy in domain composi-

### Table 1. Mouse Ubiquitination-Associated Proteins From Three Different Data Sets, Categorized by InterPro Domains and Enzyme Class

<table>
<thead>
<tr>
<th>Class</th>
<th>InterPro acc</th>
<th>RTPS</th>
<th>SPTR</th>
<th>SPTRENS</th>
<th>InterPro domain name</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>IPR000594</td>
<td>12 (4)</td>
<td>8</td>
<td>9</td>
<td>UBA/THIF-type NAD/FAD binding fold</td>
</tr>
<tr>
<td></td>
<td>IPR000127</td>
<td>6 (1)</td>
<td>6</td>
<td>7</td>
<td>Ubiquitin activating enzyme repeat domain</td>
</tr>
<tr>
<td></td>
<td>IPR000111</td>
<td>2 (0)</td>
<td>4</td>
<td>4</td>
<td>Ubiquitin-activating enzyme</td>
</tr>
<tr>
<td>E2</td>
<td>IPR000608</td>
<td>40 (13)</td>
<td>24</td>
<td>48</td>
<td>Ubiquitin-conjugating enzymes</td>
</tr>
<tr>
<td>E3</td>
<td>IPR001810</td>
<td>56 (21)</td>
<td>34</td>
<td>53</td>
<td>F-box</td>
</tr>
<tr>
<td></td>
<td>IPR001373</td>
<td>9 (5)</td>
<td>2</td>
<td>4</td>
<td>Cullin</td>
</tr>
<tr>
<td></td>
<td>IPR000569</td>
<td>25 (5)</td>
<td>14</td>
<td>33</td>
<td>HECT domain (ubiquitin-protein ligase)</td>
</tr>
<tr>
<td></td>
<td>IPR003126</td>
<td>3 (1)</td>
<td>1</td>
<td>7</td>
<td>Zinc-finger in N-recognin (putative)</td>
</tr>
<tr>
<td></td>
<td>IPR001841</td>
<td>192 (61)</td>
<td>143</td>
<td>244</td>
<td>Zinc-finger, RING</td>
</tr>
<tr>
<td></td>
<td>IPR001232</td>
<td>2 (0)</td>
<td>2</td>
<td>3</td>
<td>SKP1 component</td>
</tr>
<tr>
<td></td>
<td>IPR003613</td>
<td>8 (4)</td>
<td>3</td>
<td>4</td>
<td>Zinc-finger, modified RING (U-box)</td>
</tr>
<tr>
<td>E1</td>
<td>IPR001578</td>
<td>292 (97)</td>
<td>287</td>
<td>431</td>
<td>Zinc-finger, modified RING (U-box)</td>
</tr>
<tr>
<td>DUB</td>
<td>IPR001394</td>
<td>5 (0)</td>
<td>5</td>
<td>5</td>
<td>Ubiquitin C-terminal hydrolase, family 1</td>
</tr>
<tr>
<td></td>
<td>IPR001607</td>
<td>10 (2)</td>
<td>8</td>
<td>19</td>
<td>Ubiquitin thiolesterase, family 2</td>
</tr>
<tr>
<td></td>
<td>IPR000626</td>
<td>48 (12)</td>
<td>38</td>
<td>70</td>
<td>Ubiquitin domain</td>
</tr>
<tr>
<td></td>
<td>IPR000449</td>
<td>38 (9)</td>
<td>32</td>
<td>60</td>
<td>Ubiquitin-associated (UBA) domain</td>
</tr>
<tr>
<td></td>
<td>IPR001012</td>
<td>11 (6)</td>
<td>6</td>
<td>11</td>
<td>UBX domain</td>
</tr>
<tr>
<td></td>
<td>IPR003892</td>
<td>8 (1)</td>
<td>6</td>
<td>10</td>
<td>CUE domain</td>
</tr>
<tr>
<td></td>
<td>IPR003903</td>
<td>15 (3)</td>
<td>12</td>
<td>16</td>
<td>Ubiquitin interacting motif (UIM)</td>
</tr>
<tr>
<td></td>
<td>IPR004854</td>
<td>1 (0)</td>
<td>1</td>
<td>1</td>
<td>Ubiquitin fusion degradation protein UFD1</td>
</tr>
</tbody>
</table>

The numbers of novel proteins, not present in the other sets, are indicated in parentheses. The N/A class contains proteins possessing domains that do not yet have well understood roles in the other four classes. RTPS indicates Representative Transcript and Protein Set; SPTR, SWISS-PROT and TREMBL databases; and SPTRENS, SWISS-PROT, TREMBLE, and mouse Ensembl databases.
tion between proteins within a class; this trend is clearest within E1 and E2 proteins. The most noticeable exceptions to this are human and *C. elegans* E3 proteins, in which the various non-UA domains are distributed among more than 2.5 times the number of E3 proteins. More modest levels of redundancy in domain composition are seen within E3 proteins from every other organism except *S. cerevisiae*, within DUB proteins from most organisms and within human and mouse N/A proteins. The full domain architecture for any protein encoded by a FANTOM2 clone can be examined in the online FANTOMDB annotation database (http://fantom2.gsc.riken.go.jp/db/), which can be searched with InterPro domain accession numbers.

Eight non-UA domains were found to be present within UA proteins from every species examined: NAD binding site (IPR000205), RNA-binding region RNP-1 (IPR000548; found in a variety of RNA binding proteins), sevenfold repeat in clathrin and VPS proteins (IPR000547; may be involved with endocytosis), zinc-finger, C-x8-C-x5-C-x3-H type (IPR000571; involved in cell cycle–or growth phase–related regulation and splicing), zinc-finger C2H2 type (IPR000822; responsible for RNA and DNA binding), TPR repeat (IPR001440; a protein–protein interaction motif), G protein-β WD-40 repeat (IPR001680; present in signal transduction proteins), and zinc-finger cysteine-rich C6HC (IPR002867; function unknown). All of these domains are found in combination with E3 proteins containing either zinc-finger RING (IPR001841) or F-box (IPR001810) domains within every species. In addition, within the human proteome, two of these non-UA domains (IPR000822, IPR001440) have been recruited to ubiquitin thiolesterase family 2 (IPR001394) containing DUBs. A further four non-UA domains were found within UA proteins from every multicellular organism studied but at the same time were absent from UA proteins from the two yeast species: zinc-finger ZZ type (IPR000433), basic helix-loop-helix dimerization domain (IPR001394), tyrosine protein kinase.

<table>
<thead>
<tr>
<th>Class</th>
<th><em>Homo sapiens</em></th>
<th><em>Mus musculus</em></th>
<th>Drosophila melanogaster</th>
<th>Caenorhabditis elegans</th>
<th>Schizosaccharomyces pombe</th>
<th>Saccharomyces cerevisiae</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E1</strong></td>
<td>14 (9)</td>
<td>13 (16)</td>
<td>11 (8)</td>
<td>9 (7)</td>
<td>9 (6)</td>
<td>8 (4)</td>
</tr>
<tr>
<td></td>
<td>7 (4)</td>
<td>8 (4)</td>
<td>3 (4)</td>
<td>4 (3)</td>
<td>3 (3)</td>
<td>3 (2)</td>
</tr>
<tr>
<td></td>
<td>2 (4)</td>
<td>4 (1)</td>
<td>7 (1)</td>
<td>3 (1)</td>
<td>7 (2)</td>
<td>6 (2)</td>
</tr>
<tr>
<td><strong>Total E1</strong></td>
<td>16 (16)</td>
<td>11 (9)</td>
<td>9 (7)</td>
<td>4 (3)</td>
<td>9 (6)</td>
<td>8 (4)</td>
</tr>
<tr>
<td><strong>E2</strong></td>
<td>53 (6)</td>
<td>61 (32)</td>
<td>32 (28)</td>
<td>25 (24)</td>
<td>14 (15)</td>
<td>15 (10)</td>
</tr>
<tr>
<td></td>
<td>78 (74)</td>
<td>74 (43)</td>
<td>34 (33)</td>
<td>434 (344)</td>
<td>13 (14)</td>
<td>14 (15)</td>
</tr>
<tr>
<td><strong>Total E2</strong></td>
<td>10 (9)</td>
<td>7 (7)</td>
<td>10 (7)</td>
<td>4 (3)</td>
<td>5 (5)</td>
<td>5 (5)</td>
</tr>
<tr>
<td><strong>E3</strong></td>
<td>385 (305)</td>
<td>305 (212)</td>
<td>121 (100)</td>
<td>170 (153)</td>
<td>51 (40)</td>
<td>40 (30)</td>
</tr>
<tr>
<td></td>
<td>38 (38)</td>
<td>36 (6)</td>
<td>6 (6)</td>
<td>5 (5)</td>
<td>6 (6)</td>
<td>5 (5)</td>
</tr>
<tr>
<td><strong>Total E3</strong></td>
<td>423 (343)</td>
<td>341 (278)</td>
<td>127 (107)</td>
<td>175 (158)</td>
<td>57 (46)</td>
<td>45 (35)</td>
</tr>
<tr>
<td><strong>DUB</strong></td>
<td>14 (14)</td>
<td>14 (14)</td>
<td>32 (32)</td>
<td>33 (33)</td>
<td>16 (16)</td>
<td>16 (16)</td>
</tr>
<tr>
<td><strong>Total DUB</strong></td>
<td>17 (17)</td>
<td>17 (17)</td>
<td>40 (40)</td>
<td>43 (43)</td>
<td>18 (18)</td>
<td>18 (18)</td>
</tr>
<tr>
<td><strong>N/A</strong></td>
<td>527 (442)</td>
<td>442 (189)</td>
<td>189 (65)</td>
<td>82 (68)</td>
<td>20 (20)</td>
<td>20 (20)</td>
</tr>
<tr>
<td><strong>Total N/A</strong></td>
<td>544 (459)</td>
<td>459 (198)</td>
<td>198 (73)</td>
<td>82 (68)</td>
<td>20 (20)</td>
<td>20 (20)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>835 (764)</td>
<td>764 (320)</td>
<td>320 (785)</td>
<td>785 (162)</td>
<td>162 (145)</td>
<td>145 (125)</td>
</tr>
</tbody>
</table>

The N/A class contains proteins possessing domains that do not yet have well understood roles in the other four classes.
within zinc-finger RING (IPR001841) proteins from the En-
and the fibronectin type III repeat (IPR003962) are absent
(IPR001841). Both the butyrophilin-like (IPR003879) domain
in combination within the zinc-finger RING domain
type III repeat (IPR003962). All four of these domains appear
(IPR001660), butyrophilin-like (IPR003879), and fibronectin
mains.

Only four non-UA domains were found to be specific to
mouse and human UA proteins: HMG-I and HMG-Y DNA-
binding domain (IPR000637), sterile α motif SAM
(IPR001660), butyrophilin-like (IPR003879), and fibronectin
type III repeat (IPR003962). All four of these domains appear in
combination within the zinc-finger RING domain
(IPR001841). Both the butyrophilin-like (IPR003879) domain and
the fibronectin type III repeat (IPR003962) are absent
within zinc-finger RING (IPR001841) proteins from the En-
semb Fugu Database.

**DISCUSSION**

This analysis suggests that, as stated previously (Von Armin
2001; Jones et al. 2002), there may be a direct relationship
between the number of E2 proteins and developmental
complexity, but also that a similar case can be made for the numbers
of proteins occupying the other enzyme classes. It is clear
that most evolutionary innovation in UA proteins has oc-
curred within E3 proteins, particularly those containing zinc-
finger RING (IPR001841), F-box (IPR001810), or HECT
(IPR000569) domains. However, lineage-specific explosions
in E3 numbers, as with the F-box (IPR001810)—containing
proteins in *C. elegans*, may obscure the real relationship be-
tween the numbers of proteins in an enzyme class and an
other parameter such as developmental complexity. In addi-
tion, because E3 proteins are concerned with substrate recog-
nition, one would expect to see their numbers directly related
to total proteome size, which should confound any relation-
ship with developmental complexity. It seems premature to
draw any firm conclusions about ubiquitination and develop-
ment from such comparisons. It may be more informative to
examine differences between organisms at the level of the
component domains for each class, both in terms of the num-
bers and architectures of the proteins that possess these do-

The differences in numbers of ubiquitin thiolesterase
family 2 DUBs and proteins containing the N/A class do-
 mains, such as UIM (IPR003903) and UBA (IPR000449), are
arguably the most intriguing identified here. Early vertebrate
evolution seems to have involved a substantial increase in
ubiquitin thiolesterase family 2 domain (IPR001394) DUBs,
because there are 104 in the Ensembl Fugu database (an un-
known number of these may be fragmentary predictions and/
or encoded by pseudogenes). Most DUBs seem to have roles in
reversing, proof-reading, and introducing variations in ubiq-
utination (Ben-Neriah 2002). The data here suggest that as
well as ubiquitin conjugation and ligation, the de-
ubiquitination process has also been exploited to achieve ever
more intricate regulation of proteolysis in vertebrates.
The roles of several domains examined here have yet to be fully
described, although recently some progress has been made
with the UIM (IPR003903). The UIM has been shown to be
necessary for the mono-ubiquitination of mouse protein sub-
strates, leading to their inclusion in endocytic vesicles (Polo
et al. 2002). The substrates then become cargo in intracellular
membrane trafficking pathways and an extensive UIM-
 ubiquitin-based intracellular network has been postulated on
the basis of these results (Polo et al. 2002). In this context, it
is interesting that vertebrate evolution has involved an in-
crease in UIM-containing proteins relative to other sequenced
organisms, presumably increasing the size of any associated
intracellular network. The Ensembl Fugu database contains
only up to 13 UIM-containing proteins, suggesting that the
elaboration of this network has continued since the diver-
gencc of fish and mammals. The UBA domain (IPR000449) is
likely to be a general multi-ubiquitin binding domain
(Wilkinson et al. 2001) but also seems to mediate dimeriza-
tion (Bertolaet al. 2001). The Ensembl Fugu database con-
tains up to 79 UBA containing proteins, and so, this domain
also seems to have expanded in numbers since the emergence
of vertebrates, although perhaps not since the divergence of
fish and mammals.

If one wishes to draw conclusions about the different
cellular and developmental roles that ubiquitination has
evolved to regulate, then it is instructive to study the broader
architectural differences between UA classes across different
organisms. Since the emergence of multicellular eukaryotes,
three domains seem to have been added to the repertoire of
E3 (and human DUB) proteins: the zinc-finger ZZ type do-
main (which has an unknown function), the basic helix-loop-
helix dimerization domain (which is present in transcription
factors, influencing a variety of developmental pathways),
and the ankyrin repeat (which functions as a protein–protein
interaction domain). During the same time, the tyrosine pro-
tein kinase domain (which is involved in the response of eu-
karyotic cells to external stimuli) has been partnered with the
UBA domain. Since the evolution of vertebrates, the HMG-I
and HMG-Y DNA-binding domain (which may function
in mRNA processing) and sterile α motif (which is believed to be
involved in the regulation of numerous developmental pro-
cesses) have been added to zinc-finger RING domain E3 pro-
teins. At a later stage in vertebrate evolution, after the diver-
gencc of fish and mammals, this same family of E3 proteins
has acquired the butyrophilin-like domain (which has no
known function but is found within transcription factors, ri-
bonucleoproteins, and proto-oncoproteins) and the fibronec-
tin type III repeat (which is found in a variety of cell surface
binding proteins).

Future work uncovering the cellular roles of ubiquitina-
tion that are universal across eukaryotes may benefit from
investigation of the non-UA domains found to be present
within E3 proteins from every species examined. The func-
tions of these non-UA domains cover signaling, endocytosis,
splicing, and transcriptional regulation. Some fundamental
observations can be made about the progressive integration of
ubiquitination into the life of the eukaryotic cell throughout
evolution. Several cellular processes have been linked to ubiq-
utination since the emergence of eukaryotes: the cell cycle,
RNA processing and the regulation of transcription, intracel-
ular transport, and signaling pathways. Throughout eukary-
ocic evolution, an ever more diverse variety of links have ap-
ppeared between ubiquitination and such cellular processes.
These links have predominantly been achieved through in-
novations in the architecture of three families of E3s (possess-
ing zinc-finger RING, F-box, or HECT domains) and the ubiq-
ulti thiolesterase family 2 DUBs. However, other, presently less well understood families of UA proteins (possessing UBA, UIM, and other domains) have also contributed to the diversity of these links. It would appear that the emergence of multicellular organisms and then of vertebrates have been associated with much of this innovation, which has led to novel connections with RNA processing and signaling as well as involvement in the regulation of developmental pathways. This steady increase in the cellular connectivity of ubiquitin is likely to have increased the opportunity for fine regulation of the associated cellular processes.

**METHODS**

A nonredundant, although incomplete, *Mus musculus* proteome (based upon proteins from SWISS-PROT and TrEMBL and additional peptides predicted by Ensembl) was obtained from the EBI Proteome Analysis Database (http://www.ebi.ac.uk/proteome/; Apweiler et al. 2001). The EBI data set retrieved (30,900 proteins as of August 28, 2002) was altered to remove all proteins predicted from FANTOM2 clone sequences. Some, as indicated in either the name or description fields of the protein entry. This altered EBI data set appears as SPTRENS in Table 1. This alteration allowed the real contribution of novel proteins from RTPS6 to be calculated. UA proteins from both the SPTRENS set and the RTPS6 were defined by the presence of the InterPro domains listed below. This SPTRENS set was then combined with proteins from the RTPS6 to produce the total UA *M. musculus* proteins referred to in Table 2. Matches between RTPS6 proteins and those from SPTRENS of ≥98% identity over ≥20 residues were assumed to indicate that both sequences originated from the same gene. This may underestimate the actual number of genes if the data set contains many recent paralogs. An additional *M. musculus* proteome, composed only of proteins from SWISS-PROT and TrEMBL (SPTR in Table 1), was also retrieved from the EBI Proteome Analysis Database, to estimate the relative success of novel gene identification strategies based upon genomic or transcribed sequence. At the same time (August, 2002), nonredundant protein sets for *Homo sapiens* (33,451 protein entries derived from SWISS-PROT, TrEMBL and Ensembl), *C. elegans* (20,046 proteins), *D. melanogaster* (15,253 proteins), *S. pombe* (6206 proteins), *F. rubripes* (20,046 proteins), and *S. cerevisiae* (6206 proteins) proteomes were also retrieved from the same source. The methods used to reduce redundancy vary between the proteome sets referred to here. The FANTOM2 RTPS6 set was constructed by using mapping to the genome to reduce redundancy, and when a finished genome sequence is available, the EBI follows an analogous strategy. However, when a finished genome sequence is not available (for mouse and human sets), mapping to the genome sequences cannot always provide a definitive answer. Consequently, the EBI uses another method of reducing redundancy: liberal clustering of all proteins sharing sequence similarity, with each cluster treated as a single database entry. These strategies are discussed in detail at the EBI Proteome Analysis Database site (http://www.ebi.ac.uk/proteome/). All data sets used in this study are available from http://www.hgu.mrc.ac.uk/Users/ Colin.Semple/lab_data.html.

Given the incomplete nature of the sequence data for some of these organisms and the degree of error inherent in gene prediction, each of these data sets would be expected to contain a number of incomplete fragmentary protein sequences. It is not a trivial task to derive accurate estimates of these numbers. However, to assess the scale of this problem, the proportions of proteins from each proteome indexed to be fragmentary (within the database name or description fields) were recorded as follows: *H. sapiens*, 0.12; *M. musculus*, 0.09; *D. melanogaster*, 0.01; *C. elegans*, 0.001; *S. pombe*, 0.05; and *S. cerevisiae*, 0.01. This indicates that fragmentary proteins are most prevalent in *H. sapiens* and *M. musculus* data, and the equivalent proportions for UA proteins in these species are 0.13 and 0.05, respectively. One may conclude that some caution should be exercised in interpreting comparisons involving these two proteomes. Some comparisons involved predicted proteins from the recently sequenced draft genome sequence of the puffer fish *Fugu rubripes* (Ensembl Fugu Database Release 8.1.1; http://www.ensembl.org/Fugu_rubripes/). Given the lack of transcribed sequence data for *F. rubripes* and the unfinished state of its genome sequence, these predicted proteins should also be regarded as potentially incomplete, fragmentary and/or artificial.

The UA InterPro domains examined were as follows: 1. the three E1 domains: UBA/THIF-type NAD/FAD binding fold domain (IPR0005594), E1 replicated domain (IPR000127), and E1 domain (IPR00011); 2. the single E2 domain: E2 domain (IPR000608); 3. the seven E3 domains: SKP1 component domain (IPR001232), F-box domain (IPR01810), Cullin domain (IPR001373), HECT domain (IPR000569), zinc-finger (putative), N-recogin domain (IPR003126), zinc-finger RING domain (IPR001841), and zinc-finger-modified RING domain (also called Ubox; IPR003613); 4. the three DUB domains: ubiquitin C-terminal hydrolase family 1 domain (IPR001576), ubiquitin thiolesterase family 2 domain (IPR001394), zinc-finger in ubiquitin thiolesterase domain (IPR001607); 5. six other domains (assigned to the N/A class because other classes are not applicable at present), for which we lack a precise knowledge of their roles in ubiquitination, were also included: ubiquitin domain (IPR000626; a number of proteins contain ubiquitin-like domains with unknown functions that are similar to the ubiquitin molecule itself), UBA domain (IPR000449; recently shown to bind multi-ubiquitin chains), UBX domain (IPR001012; function unknown but may be involved in apoptosis), CUE domain (IPR003892; may be involved in binding E2s), ubiquitin interacting motif (IPR003903; involved with mono-ubiquitination of substrates leading to endocytosis), and ubiquitin fusion degradation protein UFD1 (IPR004854; involved in the recognition of ubiquitin–protein conjugates leading to degradation).

Unless otherwise stated, the functional description of these domains is derived from the appropriate InterPro entries. Con temporaneous references for each domain can also be viewed at the InterPro site (http://www.ebi.ac.uk/interpro/index.html). It is important to note that InterPro assignments are not infallible, and occasionally, a single domain may be annotated as belonging to two different categories; an example encountered in these data was completely overlapping predictions of RING finger (IPR001841) and PHD finger (IPR001965) domains. Annotation for all proteins of interest can be viewed in its entirety using the graphical annotation viewer provided by FANTOMDB (http://fantom2.gsc.riken.go.jp/db/).

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