**RING domains**

The RING domain was originally described by Freemont (ref, CHECK) as a commonly occurring motif with a characteristic pattern of cysteine and/or histidine residues. <more>. Structural studies have revealed that these cysteine and histidine residues interact with two zinc ions to form a characteristic folded structure. RING domains are highly prevalent, with other 300 RING domain containing genes in the human genome (ref 33: Li 2008, CHECK).

Consists of a core domain between 40-70 residues, with a ‘cross-brace’ pattern of conserved cysteine and histidine residues that interact with two zinc ions to form a characteristic folded structure.

**Ubiquitination pathway**

Timeline

Description automatically generated

The most commonly associated role of RING domains is in the ubiquitination pathway, the mechanism by which ubiquitin molecules are attached to substrate proteins. This pathway involves three classes of enzymes which catalyse three steps: ubiquitin-activation (E1), ubiquitin-conjugation (E2), ubiquitin-ligation (E3), the latter of which is commonly attributed to RING domain proteins. E1 activates ubiquitin and transfers to an E2, where a thioester bond is formed between the E2 and Ub. E3s then simultaneously interact with a substrate and the E2~Ub conjugate to catalyse the transfer of Ub to a target lysine on the substrate.

A single E1 works with fewer that 40 E2s (in humans), which work with hundreds of E3s. Each E3 typically interacts with just a few substrates. By acting as a bridge between the E2 and a specific substrate, RING E3s are responsible for conferring specificity to the ubiquitination reaction.

RING E3 ligases function by simultaneously binding to the E2 and the substrate and directly catalysing the transfer of ubiquitin from the E2 to the substrate (refs: see Rojas). RING E3s work by stabilising the closed conformation of the E2~Ub conjugate. This alters the geometry of the thioester bond between Ub and E2, increasing reactivity towards a substrate lysine residue. The target lysine is often determined by proximity. At the same time as stabilising the closed E2~Ub state, most RING E3 interact with a substrate to bring a target lysine into close proximity of the E2, typically via other parts of the E3 protein (e.g. TRIMs use C-terminal substrate recognition domains). Alternatively, the E3 itself can act as a substrate (autoubiquitination)

**RINGs as E3s**

It is thought that many RING domain proteins (if not most) possess this activity (Deshais, CHECK). Of those that do not possess intrinsic E3 activity, many of these interact with other RING E3s, acting as a subunit of a larger oligomeric complex (e.g. Bard1, Bmi1, MdmX).

The majority of RING E3s are constitutively active when studied in vivo, but may require regulation by post-translational modifications in vivo

**RING- E2 interaction**

RINGs appear to employ a variety of strategies to engage with E2s.

Many RINGs interact with E2s via a hydrophobic cleft on their surface. Many RINGs have conserved hydrophobic residues at these sites. Mutation of sites in this region have been shown to disrupt E2 binding and ubiquitination activity for some RING domains (e.g. c-Cb1) but not others (Brca1).

A key site in many RING E3 ligases is an arginine or lysine residue immediately downstream of the final zinc-coordinating cysteine, which is known as the linchpin site. Typically, the choice of residue at this site regulates a trade-off between ubiquitination activity and E2 specificity. RINGs with a K at this site typically show lower ubiquitination activity in vitro (REF). Stewart showed for the protein <> that mutating this site from an arginine to a lysine increases ubiquitination activity but reduces E2 specificity (CHECK). 46% of RING domains have an arginine at this site linchpin, whereas 14% have lysine (Stewart). Other RING domains have other residues at this site, some of which have been shown to have ubiquitination activity despite this <examples, refs>. This suggests that the linchpin mechanism isn’t universal to all RING domains, and other mechanisms of <> must exist. Currently this is poorly understood.

Because of this variety, we are not at the stage where we can unambiguously predict how/whether a RING will interact with E2s, or the specific E2 to which a RING will bind.

The choice of E2 often determines the type of ubiquitination that occurs (i.e. mono vs poly, or type of poly connection), which is directly related to the outcome of ubiquitination.

**Functions of ubiquitination**

Substrates can be monoubiquitinated at a target lysisne, or polyubiquitinated, meaning the formation of a ubiquitin chain via lysine molecules on ubiquitin monomers. Monoubiquitination is associated with modification of protein function. <>. Depending on the type of linkage, polyubiquitination can also be associated with modification of protein function, but is most commonly associated with proteasome-dependent proteolysis.

Many E3s are known to be autoubiquitinated. In many cases this may be a side effect of E3 activity with no functional role, but in some cases this may serve a purpose. Autoubiquitination of BCA2 has been shown as an elegant mechanism to regulate steady state levels of the protein (Amemiya). Autoubiquitination is competitive with substrate inhibition, allowing it to regulate protein levels according to substrate availability. Other examples in the review.

**Dimerisation**

**Table

Description automatically generated with low confidence**

**Diagram

Description automatically generated**

Table of deltaG values from the paper

Many RING domains have been shown to self-associate in crystal structures, forming dimers or higher order oligomers. In most cases, dimerisation is achieved via hydrophobic interactions between short alpha-helical segments at the N and C termini of the core RING domain, which forms a four-helix bundle involving two RING monomers. This is stabilised by additional contacts in the core RING domain.

In solution, the oligomeric state of these dimeric RINGs varies dramatically, from monomeric to constitutively dimeric. <more>

TRIM21 exists in a monomer-dimer equilibrium in solution (Dickson)

TRIM32 appears constitutively dimeric in solution (Koliopoulos)

TRIM25 crystalises as a dimer, but appears entirely monomeric in solution, and dimerisation is only detectable at very high concentrations (Koliopoulos).

Deletion of the N- and C- terminal helices, or mutation of key hydrophobic residues, has been shown for many RINGs to eliminate dimerization and produce an entirely monomeric form of the protein.

Notably, however, not all RING domains dimerise. The TRIM28 RING domain has a small N-terminal helix, but lacks a C-terminal helix, and forms no dimerisation contacts in crystal structures (ref). The TRIM56 RING domain lacks both helices, and similarly is monomeric in crystal structures (ref). The TRIM19 RING on the other hand, forms a ‘torus-shaped’ tetramer in crystal structures.

**Induced dimerisation**

In many cases dimerisation is context specific. RNF4 is largely monomeric at physiological concentrations in vitro, but is able to dimerise upon addition of its substrate. Dual binding of two RNF4 molecules to a single substrate molecule creates a locally high concentration of RNF4 that permits dimerisation (Rojas-Fernandez). Similarly, TRIM25 shows weak dimerisation ability on its own, but is stabilised by binding to an E2.

**Functions of dimerisation**

In many cases the significance of dimerisation is fundamentally linked to the ubiquitination role of RINGs. Dimerisation stabilises the closed E2-Ub conformation, by allowing each Ub to make simultaneous contacts with two RING domains. In many cases this is essential for ubiquitination activity. Mutations to key hydrophobic residues in the helices flanking the core RING domain disrupt dimerisation and reduce ubiquitination activity. On the other hand, forced dimerisation of RINGs can hyperactivate ubiquitination activity, as has been shown for RNF4 (ref).

In many RING E3s, intrinsic RING dimerisation is supplemented by additional dimerisation domains in the protein (e.g. coiled coil domains for TRIM RING E3s), in some cases leading to higher order assemblies.

**Monomeric RING domains**

Some RING E3s do not require dimerisation to function. In these cases, E2~Ub stabilisation often requires additional contacts outside the RING domain. For example, in the CBL class of RING domains, a phosphorylated tyrosine residue outside of the core RING domain interacts with Ub, which synergises with the RING domain to stabilise the E2~Ub in a similar manner to a dimeric RING (Dou)