**RING domains**

The RING domain was originally described by Freemont (ref, CHECK) as a commonly occurring motif, between 40 and 70 residues in length, with a characteristic pattern of cysteine and/or histidine residues. Structural studies have revealed a ‘cross-brace’ pattern, whereby 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).

**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 (fig x): 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. The choice of E2 often determines the type of ubiquitination that occurs. This can be either monoubiquitination, where a single ubiquitin molecule is added to a target lysine, or polyubiquitination, meaning the formation of a ubiquitin chain via lysine molecules on ubiquitin monomers. The functional consequences of ubiquitination are directly related to the type of ubiquitination. Monoubiquitination is associated with modification of protein function. <more>. 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. Each E3 typically interacts with only a small number of E2s, and a small number of substrates. Therefore, by acting as a bridge between a specific E2 and a specific substrate, E3s are responsible for conferring specificity to the ubiquitination reaction.

It is thought that many RING domain proteins (if not most) possess E3 ligase activity (Deshais, CHECK), catalysing the transfer of Ub directly from an E2 to a target substrate. RING E3s work by binding to and stabilising the closed conformation of an E2~Ub conjugate. This alters the geometry of the thioester bond between Ub and E2, increasing reactivity towards a substrate lysine residue. At the same time as interacting with the E2, RING E3s bind to a substrate, bringing a specific lysine on the substrate into close proximity of the E2. This then leads to direct transfer of Ub from the E2 to the substrate. Typically, this E3/substrate interaction is via other parts of the E3 protein, rather than the RING domain itself (e.g. TRIMs use C-terminal substrate recognition domains).

Alternatively, lysines on the E3 itself can be targeted. 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). As autoubiquitination is competitive with substrate ubiquitination, the protein is able to regulate its own levels according to substrate availability. <Other examples in the review>.

For later / discussion

Many RING E3s show constitutive ubiquitination activity when studied in vivo, although in vivo they may require regulation by post-translational modifications. Of the handful of RING domains that have been shown not to possess intrinsic E3 activity, many of these are known to interact with other RING E3s, acting as a subunit of an active oligomeric complex (e.g. Bard1, Bmi1, MdmX).

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).

**Dimerisation**

**Table

Description automatically generated with low confidence**

**Diagram

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Table of deltaG values from the paper

Another key function attributed to RING domains is their ability to act as dimerisation domains. Many purified 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 (fig x). This is stabilised by additional contacts in the core RING domain.

However, whilst the majority of RING domains are dimeric in crystal structures, the oligomeric state in solution varies dramatically, ranging from entirely monomeric to constitutively dimeric. For example, whereas TRIM32 appears constitutively dimeric in solution (Koliopoulos), TRIM21 exists in a monomer-dimer equilibrium (Dickson), whereas TRIM25, whilst crystalising as a dimer, appears entirely monomeric in solution, only dimerising at very high concentrations (Koliopoulos). <comment on this, related to interface size?, deltaG?>.

In many cases the significance of dimerisation is fundamentally linked to the ubiquitination role of RINGs. RING 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 the case of RING domains at the weaker end of the dimerisation scale, which exist largely as monomers in isolation, the ability to dimerise may be 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). <comment on this> Similarly, TRIM25 shows weak dimerisation ability on its own, but is stabilised by binding to an E2.

Notably, however, not all RING domains dimerise. Whereas some form higher order structures (e.g. the TRIM19 RING, which forms a ‘torus-shaped’ tetramer in crystal structures), others lack any ability to oligomerise. The TRIM28 RING domain, for example, 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). Likewise, the CBL class of RING domains <more>. In the absence of dimerisation, 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).

Given the widespread roles of dimerisation <>, it’s not unlikely that RING domains have evolved functions as dimerisation domains in other contexts unrelated to ubiquitination….

For discussion:

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.