**Amemiya, 2008**

Autoubiquitination regulates steady state levels of protein. Autoubiquitination is competitive with substrate inhibition, allowing it to regulate protein levels according to substrate availability

**Liew, 2010**

Shown to be dimeric by SEC MALS. Mutations at dimerisation interfaces make it monomeric. Ubiquitination activity diminished. RNF4

This suggests that C terminal region isn’t involved in dimerisation, but this may be outdated. Rojos-fernandez pepr suggests that it is

**Pruneda, 2012**

Linchpin. Seems to be the original observation

**Scott, 2014**

More linchpin

**Rojas-Fernandez, 2014**

RNF4, whilst largely monomeric at physiological concentrations in vitro, but is able to dimerise upon addition of its substrate. Dual binding off RNF4 to its substrate creates a locally high concentration that permits dimerisation.

Kd in the absence of SUMO is around 200nM, far greater than the estimated intracellular concentration of RNF4.

Dimerisation mutants have no ubiquitination activity

Forced dimerisation of the RING domain hyperactivates ubiquitination

**Koliopoulos, 2016**

TRIM32 RING domain can dimerise, which is dependent on the alpha-helical sequences flanking the core RING domain which form a 4-helix bundle. References Brzovic 2001as the original descriptor of this type of dimerisation.

TRIM32 RING is only active as a dimer

TRIM25 crystalises as a dimer, but appears monomeric in SEC MALS. Very low affinity, even at concentrations as high as 100uM no significant dimerisation can be observed. Mutation to the dimerisation interface disrupts catalytic activity. Dimerisation is likely enhanced in the context of the full length protein, or by interaction with the E2

Dimerisation of TRIM25 is only detectable at high protein concentrations

**Stewart, 2017**

Mutation of the linchpin from K to R increases ubiquitination activity (and reduces E2 specificity?)

46% of RING domains have an Arg as linchpin, 14% have lysine

**Dickson, 2018**

TRIM21 exists in a monomer-dimer equilibrium, which can be disrupted by mutation

**Stevens, 2019**

Key reviews:

Deshaies, 2009

Metzger, 2014

Fiorentini, 2020

**Basics of ubiquitination pathway**

**Intrinsic dimerisation/oligomerisation activity**

Dimerisation stabilises the closed E2-Ub conformation.

Many RING domains have been shown to self-associate via hydrophobic interactions between short alpha-helical segments at the N and C termini off the core RING domain, which forms a four-helix bundle. This is stabilised by additional contacts in the core RING domain.

However, the oligomeric state in solution varies dramatically

**Dimerisation interface mutants**

**Interaction with E2s**

RING E3 ligases function by simulataneously binding to the E2 and the substrate and directly catalysing the transfer of ubiquitin from the E2 to the substrate (refs: see Rojas)

**Autoubiquitination**

**Linchpin**