Calpains cleave dysferlin to release a synaptotagmin-like module for the calcium-dependent exocytosis of membrane repair

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The ability to repair a plasma membrane injury is an intrinsic property of eukaryotic cells. Membrane repair is calcium-dependent, and thought to involve the aggregation and fusion of vesicles at the site of injury, forming a "patch" to seal ruptures in the cell membrane, with mechanistic parallels with synaptic exocytosis.

Dysferlin belongs to an ancient family of vesicle fusion proteins, with the unique feature of seven tandem C2 domains, calcium-regulated lipid binding domains. Mutations within the Dysf gene cause a form of inherited muscular dystrophy, and dysferlin-deficient muscle fibres are unable to reseal an acute plasma membrane injury. Thus it is proposed that dysferlin mediates the vesicle fusion of membrane repair.

We show that with membrane injury, activated calpains cleave dysferlin to release a C-terminal module we termed mini-dysferlin$_{C72}$. Our results suggest it is the cleaved mini-dysferlin$_{C72}$ that is specifically recruited and undergoes calcium-dependent vesicle fusion at injury sites (Lek *et al.*, 2013). Calpain cleavage of dysferlin is mediated by the ubiquitous calpains, via a cleavage motif encoded by an alternately spliced exon, exon 40a (Redpath *et al.*, 2014).

Importantly, we reveal other members of the ferlin family are also cleaved enzymatically to release similar C-terminal modules, bearing two C2 domains and transmembrane domain, with structural similarity to the classical mediators of synaptic vesicle fusion, the synaptotagmins. Our results suggest that calpain-cleavage of ferlins presents an ancestral means to release synaptotagmin-like effector modules for vesicle fusion where and when calcium signaling demands, and for dysferlin, this includes the calcium-activated vesicle fusion of membrane repair.

Lek A, Evesson FJ, Lemckert FA, Redpath GM, Lueders AK, Turnbull L, Whitchurch CB, North KN, Cooper ST. (2013) Calpains, cleaved mini-dysferlin$_{C72}$ and L-type channels underpin calcium-dependent muscle membrane repair. *Journal of Neuroscience* **33**: 5085–5094.

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