Limited proteolysis as a tool to probe the tertiary conformation of dysferlin and structural consequences of patient missense variant L344P

Natalie Woolger1,2, Adam Bournazos1,2, Reece A. Sophocleous1,2, Frances J. Evesson1,2, Angela Lek1,3, Birgit Driemer1,2, R. Bryan Sutton3 and Sandra T. Cooper1,2

1 Institute for Neuroscience and Muscle Research, Kids Research Institute, The Children’s Hospital at Westmead, Locked Bag 4001, Westmead 2145, Australia; 2 Discipline of Child and Adolescent Health, Faculty of Medicine, University of Sydney, Australia; 3 Department of Cell Physiology and Molecular Biophysics, Texas Tech University Health Sciences Center, Lubbock, TX 79430, United States of America.

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Supplemental Data includes:

- **Supplemental Figure 1**: Identical fragmentation pattern is observed between canonical WT-DYSF isoform and Exon40a containing isoform with the exception of mini-dysferlinC72.
- **Supplemental Figure 2**: Digestion with Chymotrypsin reveals dominant in situ conformation of dysferlin following injury.
- **Supplemental Figure 3**: Masking of Hamlet-2 epitope with cells expressing patient missense variant L344P
**Supplemental Figure 1:** A) Identical fragmentation pattern is observed between canonical WT-DYSF isoform and Exon40a containing isoform with the exception of mini-dysferlinC72. Supplemental figure 1 shows representative western blot of HEK293 cells transfected with WT-DYSF lacking exon 40a (NP_003485) or dysferlin bearing exon 40a DSYF-40a (NM_001130978.1) subject to scrape harvest and limited proteolysis. Western blots were probed with the C-terminal antibody Hamlet-1, which shows an identical banding pattern of DYSF-40a to WT-DYSF, with the exception of the formation of ‘mini-dysferlinC72’ fragment released by calpain cleavage following injury. B) C-terminal tryptic fragments contain transmembrane domains and short extracellular domain. Supplemental Figure 2 shows representative western blot of HEK293 cells transfected with dysferlin bearing exon40a, subjected to scrape harvest and limited proteolysis or scrape harvest, limited proteolysis and sucrose gradient ultracentrifugation. Western blots were probed with m-Myc (mouse anti-Myc) or r-Myc (rabbit anti-Myc) to determine whether protected fragments included the Myc epitope tag at the extreme C-terminus of dysferlin. Blots show same banding pattern as Hamlet-1, suggesting extreme C-terminus is included in protected domains.
Supplemental Figure 2: Digestion with Chymotrypsin reveals dominant in situ conformation of dysferlin following injury. A) Schematic of dysferlin protein showing in silico determination of all chymotrypsin cleavage sites (black lines) and position of dysferlin antibody epitopes. B) HEK293 transfected with the DYSF40a expression construct were subjected to scrape injury 24 hrs post transfection and subject to limited proteolysis with chymotrypsin (see Methods). Digested samples were analysed by SDS-PAGE and separate western blots probed using each of the dysferlin antibodies. Schematics depict the most likely domain structure of generated chymotrypsin fragments consistently detected in n=4 proteolysis experiments, based on calculated molecular weight and immunoreactivity to dysferlin antibodies. Results are consistent with: 1) C2A excised as a solo domain. See vi - differential recognition of a 220-250 kDa doublet by Hamlet-1 and Romeo. However, the excised C2A module could not be detected with Romeo, either because it was digested by chymotrypsin, or the Romeo epitope was removed. 2) Variably digested mid-domain regions encompassing C2B-C2C-Fer-DysF.
3) the protected C-terminal module of 4 C2 domains (C_{115}). Chymotrypsin resulted in greater sub-digestion of C-terminal fragments, likely influenced by the longer required chymotrypsin digestion time (20 mins - 1 hour at 37°C) that may be complicated by progressive digestion of cleaved fragments.
Supplemental Figure 3: Masking of Hamlet-2 epitope with cells expressing patient missense variant L344P. C2C12 polyclonal cell lines stably expressing EGFP-WT-DYSF or EGFP-L344P-DYSF were harvested, fixed and permeabilised for total cell staining with the dysferlin antibodies Romeo and Hamlet-2, and subjected to flow cytometry. Representative flow data for WT-DYSF versus patient variant L344P-DYSF of GFP fluorescence versus Hamlet-2 shows masked Hamlet-2 detection for WT-DYSF. ii) Representative flow cytometry data is presented in histogram form.