NEW PROTEINS WITH PRION-LIKE DOMAINS INVOLVED IN NEURODEGENERATIVE DISEASE

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What is prion and how can we study them?

Prions are self-templating protein conformers that are naturally spread within an individual, between individuals, and even between different species and promote phenotypic change.

Yeast

The prion domain is enriched in asparagine, glutamine, tyrosine and glycine. Prion-encoded phenotypes can be beneficial, neutral or deleterious depending upon genetic background and environmental conditions.

Humans

Proteins with prion-like domains (PrLD) resemble to the ones found in yeast but preclude prionogenesis and promote progressive neurodegenerative disorders as a consequence of protein misfolding and aggregation.

Bioinformatic Tools PrionScan and PAPA + **Experimental data** → **Candidate proteins:** hnRNPA1 and Med12

Bioinformatic tools - PrionScan and PAPA algorithms

hnRNPA1	PrionScan	PAPA
Score	High score	Very high score

Med12	PrionScan	PAPA
Score	Very high score	High score

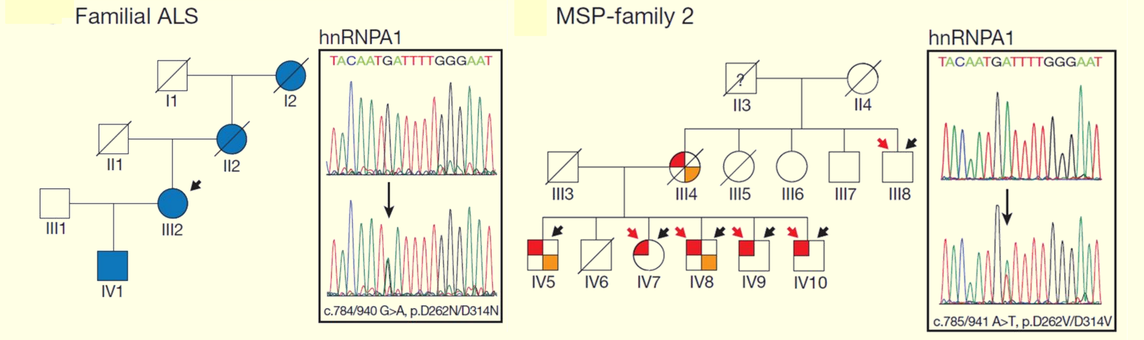
PrionScan and PAPA (*prion aggregation prediction algorithm)* are two predictive PrLD algorithms which allowed scoring protein sequences according to their likelihood of being prions. They agree that an intrinsically disordered glutamine/asparagine (Q/N) rich prion forming domain (PFD) drives prion formation.

hnRNPA1 and Med12 have high score although only PrLD-hnRNPA1 has been proved experimentally with exome sequencing of two families with neurodegenerative diseases.
Here a hypothesis for Med12 role in neurodegeneration is proposed.

hnRNPA1 - Proved role in neurodegeneration

hnRNPs comprise a multifunctional family of RNA-binding proteins. They participate in pre-mRNA processing such as splicing and are important determinants of mRNA export, localization, translation and stability.

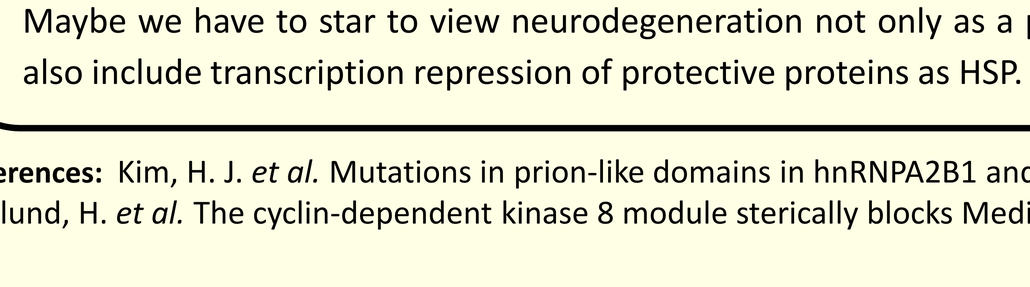
Mutations greatly accelerate hnRNA1 ability to aggregate and form amyloid fibers in both hnRNPA1 isoforms.



Amyotrophic lateral sclerosis
D262N in hnRNPA1
D314N in hnRNPA1-B

Multisystem proteinopathy
D262V in hnRNPA1
D314V in hnRNPA1-B

The critical rate-limiting step in fibrillization is **nucleation**. The disease mutations shorten the lag phase and enhance fibrillation while the WT protein remained in lag phase. Sequence alignment of hnRNPA1 orthologues show evolutionary conservation of the mutated aspartate and surrounding residues, which means is an important residue to its molecular function.



Med 12 – Hypothetical role in neurodegeneration

Med12 is one of the proteins of Mediator, a complex which serves as a bridge between DNA-binding transcription factors, G9a histone methyltransferase, and RNA polymerase II. It is composed of 4 different subunits: head, middle, tail and CDK8.

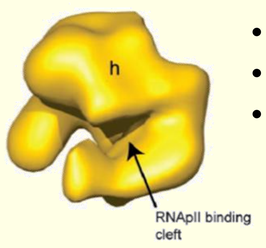
Mediator regulates transcriptional activation in the **S-Mediator active conformation**, whereas the association of separable kinase CDK8 module forms the **L-Mediator repressive conformation** blocking the interacction with RNA polimerase II. Med12 is found in this CDK8 module.

Interestingly, genome-wide analyses showed profound impact of MED12 variation on four members of HSP70 heat-shock protein expression in HEK293 cells in vitro. **Heat shock proteins** (**HSP**) are highly conserved molecular chaperones that recognize and selectively bind proteins to form stable complexes to prevent misfolding and aggregation.

Proposed hypothesis

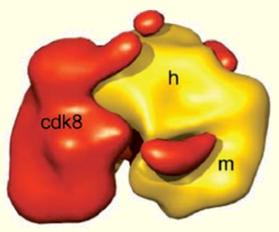
Med12 mutations
↓
Estabilizes L-Mediator conformation
↓
Repression of HSP genes transcription
↓
Less protection against misfolding
↓
More aggregation events
↓
Neurodegeneration

Mediator S-active conformation



- Head
- Middle
- Tail

Mediator L-repressive conformation



- Head
- Middle
- Tail
- CDK8 kinase

Conclusions

- PrLDs are driven by aminoacid composition rather than primary sequence.
- PrLDs are found in proteins from almost all the evolutionary classifications and taxa and in a diverse group of evolutionarily unrelated proteins.
- In a time in which prion biology is a rather unexplored field, and the number of prion proteins confirmed experimentally is scarce, predictive approaches such as PAPA and PrionScan could be of great help to pinpoint putative prionogenic proteins for further experimental characterization.
- Mutations found in PrLDs enhance proteins ability of fibrillization shortening nucleation step, which eventually causes neurodegeneration.
- Maybe we have to star to view neurodegeneration not only as a protein misfolding disease but as a group of different deregulation events which also include transcription repression of protective proteins as HSP.

References: Kim, H. J. *et al.* Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. *Nature* **495,** 467–73 (2013)
Elmlund, H. *et al.* The cyclin-dependent kinase 8 module sterically blocks Mediator interactions with RNA polymerase II. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 15788–93 (2006)