The ABCF gene family facilitates disaggregation during animal development

Sydney Skuodas, Amy Clemons, Mike Hayes, Ashley Goll, Bryan Phillips , Daniel Weeks, Jan Fassler

Departments of Biology and Biochemistry, University of Iowa

Project summary

- We find that the highly conserved ABCF gene family of non-membrane bound ATP binding cassette “transporters” facilitate disaggregation of disordered as well as ordered aggregates in the yeast, *Saccharomyces cerevisiae*.
- Members of this gene family are expressed during early stages of animal development and their knock-down affects the developmental programs of *C. elegans* and *X. laevis*.
- **Hypothesis**: Members of the ABCF gene family have an essential role as disaggregases in animals, needed for the processing of native amyloid aggregates that we find to be abundant during gametogenesis and the earliest stages of animal development.

Amyloid aggregates are present in early animal development

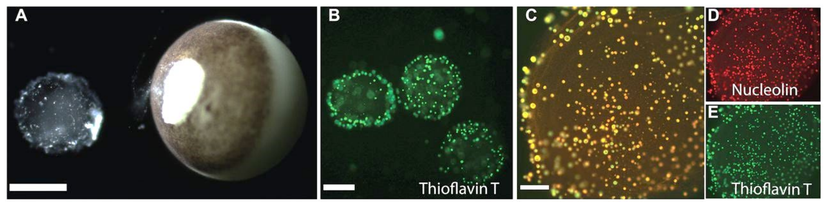


Figure 1: Amyloids are detected in isolated *Xenopus* nuclei (GVs) using Thioflavin-T, which stains amyloid fibers. (A) Manual removal of a GV from a stage VI oocyte. **(B)** Isolated GVs demonstrate amyloid containing particles seconds after Thioflavin-T staining. **(C)** Overlay of immunofluorescence of the nucleolar marker, nucleolin (red, **D**) and Thioflavin -T (green, **E**) staining in an isolated GV.

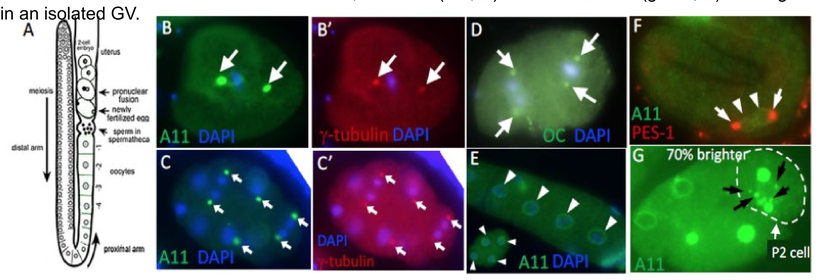
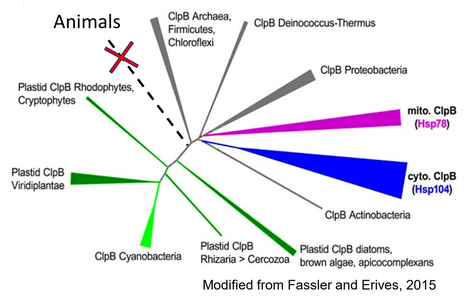


Figure 2. *A*myloids are detected in the *C. elegans* germline and during early embryogenesis using A11 antibody, which detects amyloid oligomers and OC antibody, which detects fibers. (A) Hermaphrodite gonad cartoon showing germline and early embryos **(B and C)** A11 antibody positive puncta (green, arrows) **(B’ and C’)** γ-tubulin staining (red) shows these puncta colocalize with centrosomes. **(D)** A similar pattern is also seen with the OC antibody (green). **(E,F)** A11 (green) stains nuclear membranes in germline oocytes (arrowheads), all cells in early embryos (inset arrowheads) and select cells in late embryos (arrowheads in F). Cells in **(F)** identified as primordial germ cells by the PES-1 (red) expression in the adjacent somatic gonadal precursors. **(G)** Non-centrosomal A11 puncta in the P2 cell that become visible with longer exposure (black arrows). Blue, DAPI stained DNA

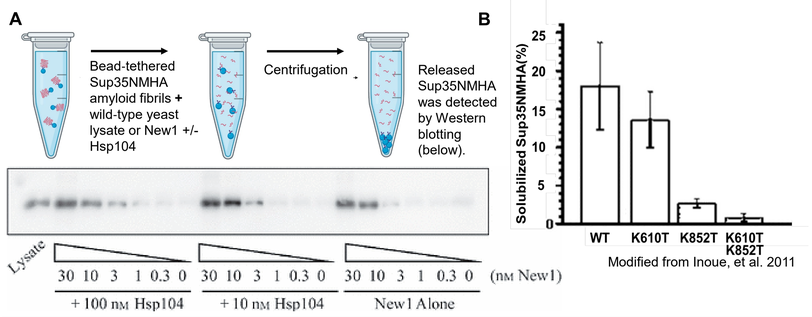
What disaggregases process amyloids in animal development?

Figure3. *HSP104* (also known as *clpB*) is a potent disaggregase conserved across all domains of life, but absent from animal genomes. An unrooted, neighbor-joining tree of the highly-conserved *clpB* genes shows that ClpB is not only conserved in all domains of life (except animals), but is also maintained in all major cellular compartments including chloroplast (plastid), mitochondria and cytosol.



Modified from Fassler and Erives, 2015

Figure 4. New1 is a fungal disaggregase that can process amyloids in the absence of Hsp104. (A) New1 is amyloid disaggregase that has Hsp104-independent activity**.** Purified New1 can break up Sup35 amyloids *in vitro.* **(B)** Amino acid substitutions (Lysine (K) to Threonine (T)) in the second ATPase domain of New1 greatly reduce its ability to solubilize Sup35NMHA amyloids.



The closest relatives to fungal *NEW1* in animals are members of the F subfamily of the ABC superfamily of genes

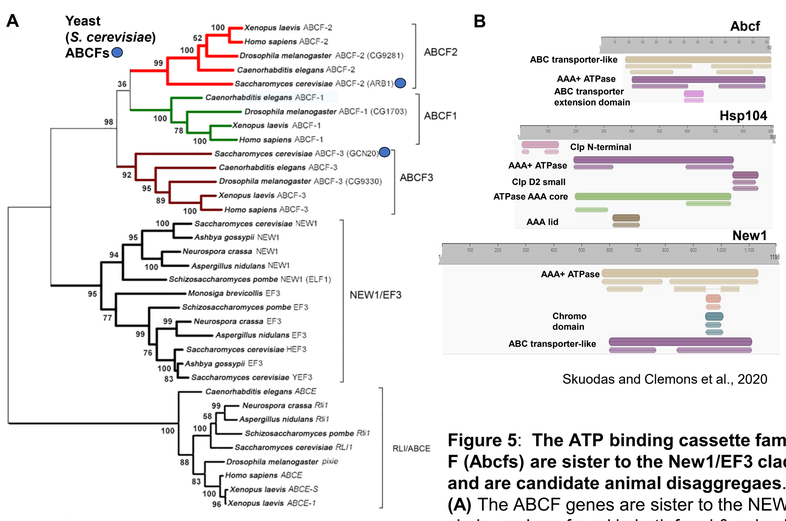


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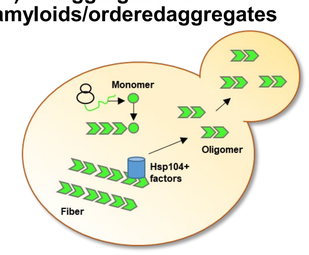
Figure 5: The ATP binding cassette family F (Abcfs) are sister to the New1/EF3 clade and are candidate animal disaggregaes. (A) The ABCF genes are sister to the NEW1 clade, and are found in both fungi & animals. Maximum likelihood phylogenetic analysis was conducted using MEGA 7.0 using protein sequences Branch length=number of substitutions/site. **(B)** AAA+ ATPase domains required for disaggregation activity in Hsp104 are also present in New1 and Abcf proteins. Unlike other ABC members, the F family has no transmembrane domain and localizes in the cytoplasm. Images were generated with InterProScan Search.

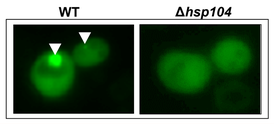
Yeast models for investigating Abcf disaggregase activity on ordered (A) and disordered (B) aggregates

(A) Disaggregation of amyloids/orderedaggregates

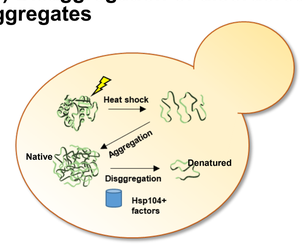


Hsp104 disaggregase activity is required for the inheritance of amyloid oligomers (seeds) from mother to daughter cell.

Disaggregase activity is indicated by cells with visible Htt-Q97-GFP aggregates

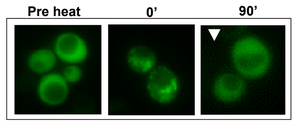


(B) Disaggregation of disordered aggregates



Hsp104 disaggregase activity is required for the resolubilization of stress-induced aggregates

Disaggregase activity is indicated by resolubilization of luciferase aggregates after heat shock



Yeast Abcf2(Arb1), Abcf3(Gcn20), and New1 are required for normal processing of Htt-Q97 amyloids

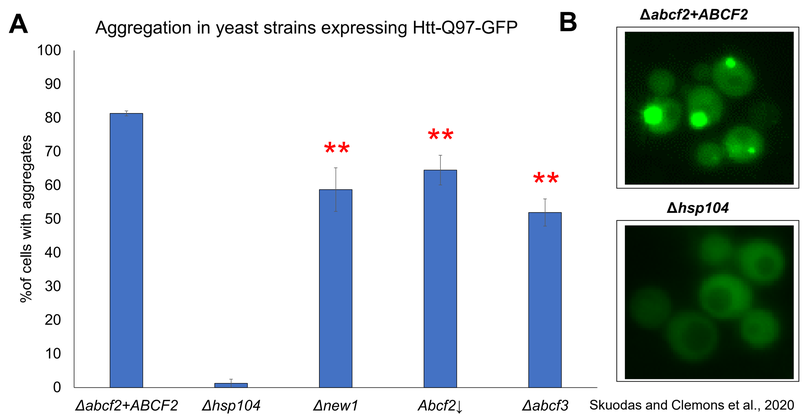


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Figure 6. Abcf2, Abcf3, and New1 are required for normal amyloid processing in *S. cerevisiae*. (A) Most *Δabcf2+ABCF2* have cells with visible Htt-Q97-GFP aggregates, whereas aggregates are absent in *Δhsp104* mutants. Abcf2 depleted cultures (Abcf2↓; ABCF2 is an essential gene) exhibit a reduced aggregate fraction relative to wild type, suggesting that Abcf2 plays a role in aggregate processing. *Δnew1* and *Δabcf3 (gcn20)* mutants exhibit a similar intermediate phenotype. **, P<0.01 by ANOVA-Tukey relative to the complemented strain. **(B)** *Δabcf2+ABCF2* cells (like wild type) typically have one large puncta and several smaller puncta, indicating multiple aggregates, while *Δhsp104* have a diffuse GFP signal, indicating soluble protein. Mutant cell populations have a reduced number of puncta, but these puncta do not differ qualitatively from the complemented strain.

Abcf2 is also required for normal disaggregation of denatured luciferase

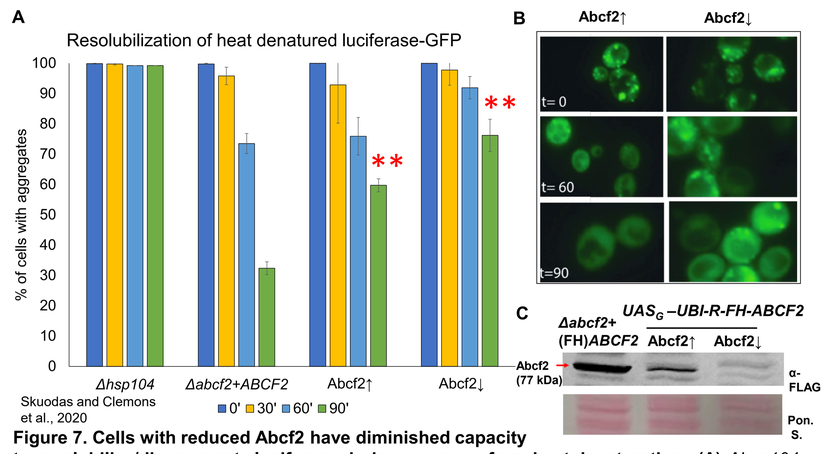

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Figure 7. Cells with reduced Abcf2 have diminished capacity to resolubilize/disaggregate luciferase during recovery from heat denaturation. (A) *Δhsp104* mutants are not able to resolubilize luciferase after heat denaturation, while most *Δabcf2+ABCF2* cells were able to resolubilize luciferase after 90 min. Cells with different levels Abcf2 depletion, (Abcf2↑ and Abcf2↓, see western **(C)**) are slower to recover after heat denaturation. **, P<0.01, ANOVA-Tukey with respect to the complemented strain. **(B)** Representative images show more cells with aggregates after 90 minutes in Abcf2 depleted cultures. **(C)** Western blot analysis showing variable levels of Abcf2 in strains containing the wild type *ABCF2* gene compared to strains reduced levels of Abcf2 protein(↑ and ↓). The level of Abcf2 correlates with the rate of luciferase resolubilization shown in **(A).**

Animal Abcf’s complement yeast aggregate processing phenotypes, likely in an ATPase dependent manner

Percent complementation

Yeast or animal Abcf clone	*Abcf2↓-Luciferase*	*Δabcf3-Htt-Q97*
Yeast *ABCF2(clone)* or *ABCF3(genomic)*	100	100
Xl-ABCF2	50	NT
Ce-ABCF2	81	NT
Hs-ABCF2	89	90
Hs-ABCF3	NS	87
Hs-ABCF1	101	103
Xl-ABCF2 mutant (K431W)	0	NT

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Chaperone activities of the ABCF proteins may be conserved

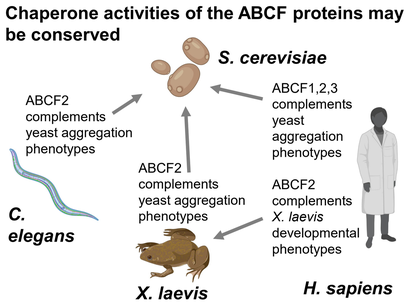
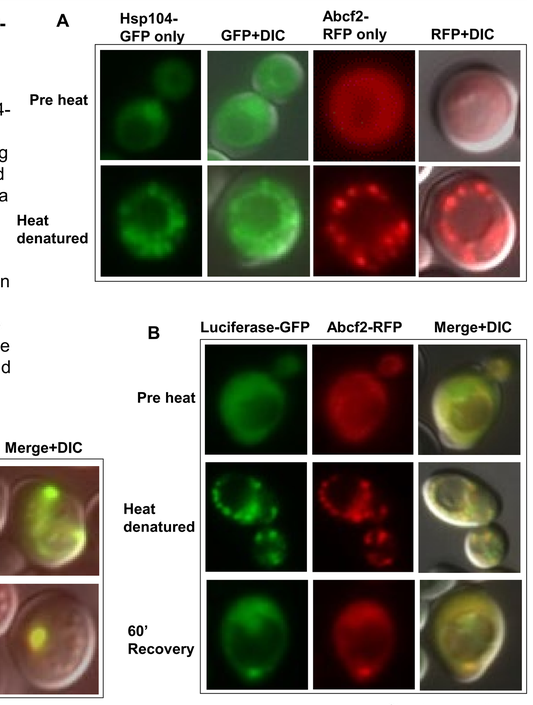


Figure 8. Animal *ABCFs* complement the luciferase-GFP and Htt-Q97-GFP phenotypes in yeast strains with reduced yeast Abcf2 and *Δabcf3.* The activity of animal ABCF family proteins were tested in yeast strains with reduced Abcf2 or with a deletion of the *ABCF3* (*GCN20*) gene. Animal *ABCF1* and *ABCF2* significantly complement the rate of luciferase disaggregation at 90 and 120 minutes. 120 minutes of recovery was used in calculating complementation values. A mutation in the Walker Motif of *Xl-ABCF2* ATPase domain eliminates any complementation. The *Δabcf3* mutant has a reduced number of cells with visible Htt-Q97-GFP aggregates. Human *ABCF1, 2, and 3* all significantly complement this phenotype (p<0.01). NT, not tested.

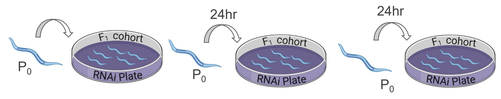
Co-localization of Abcf2 with heat-denatured aggregates and endogenous amyloids is consistent with a direct role in disaggregation

Figure 8. Co-localization of Abcf2-RFP with aggregate reporters indicates a possible direct interaction. (A) The well characterized disaggregase Hsp104-GFP forms puncta after 42°C heat shock as part of its role in interacting directly with endogenous disordered aggregates. Abcf2 also forms puncta after heat shock. **(B)** Preliminary experiments show that Abcf2-RFP co-localizes with luciferase-GFP in 100% of cells after heat shock (when both are visible in the same cell) Abcf2 co-localization with luciferase continues during recovery. **(C)** Of the rare Sup35NM-GFP fibers and round aggregates that form in log phase, ~90% overlap Abcf2-RFP.



Skuodas, unpublished

ABCF genes affect native *C. elegans* aggregates and are essential for *C. elegans* (A, B) and *X. laevis* (C) development



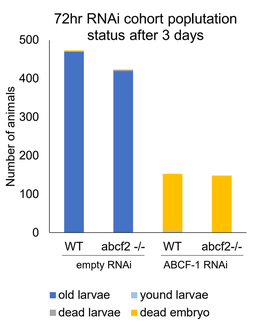
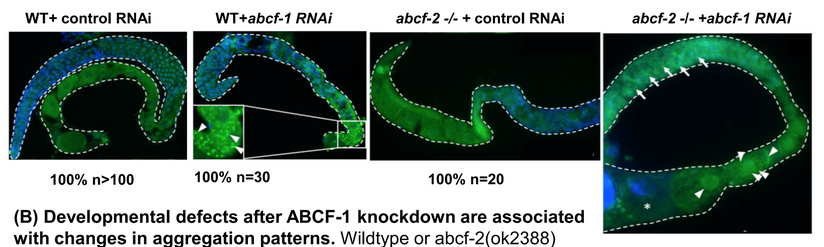
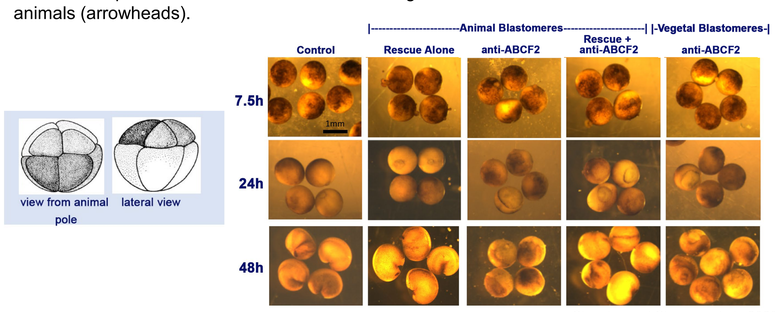


Figure 9. (A) ABCF-1 is essential for *C. elegans* development. ABCF-1 RNAi produces germline and early embryonic phenotypes. To account for delayed RNAi efficacy, parent worms were transferred to new ABCF-1 or empty vector RNAi plates every 24 hours. Progeny from each plate cohort were surveyed for developmental milestones. The effect of ABCF-1 knockdown was the strongest after the parents were on RNAi plates for 3 days. There were fewer progeny, and those that were produced died at the embryo stage, showing that ABCF1 is essential for development. There was no significant difference between WT and abcf-2(ok2388) worms



(B) Developmental defects after ABCF-1 knockdown are associated with changes in aggregation patterns. Wildtype or abcf-2(ok2388) adult germlines exposed to control or abcf1 RNAi and stained for amyloid oligomers (A11, green) and DNA (DAPI, blue). Frequencies of depicted phenotypes are indicated bellow each panel. Arrowheads denote A11 puncta. Arrows denote expanded A11 puncta in abcf-2 /abcf-1 loss of function compared to the localization in abcf-1 single loss of function animals (arrowheads).

71% (n=17) of germlines showed expansion of A11-positive structures into the distal germline. 76% (n=17) showed enlarged or fused oocytes(*)



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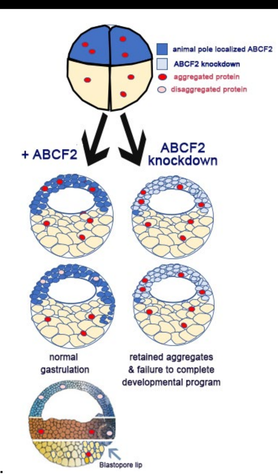
(C) Developmental phenotypes are seen after reducing endogenous ABCF2 in the animal pole and can be rescued by human ABCF2

ABCF2 is maternally inherited and localized to the animal hemisphere. Anti-*ABCF2* oligonucleotide was injected into 8-cell embryos having visually distinct animal and vegetal tiers of cell. 0.1ng of oligonucleotide, human *ABCF2* mRNA or both were injected into the 4 animal hemisphere cells or the 4 vegetal pole cells as indicated. By 24 hours the delay in cell cleavage and failure to gastrulate is visible in embryos injected with anti-ABCF2 alone. Arrows in the 24h images denote the blastopore lip.

Summary

- Abcf2 (Arb1) contributes to the disaggregation of (heat denatured) aggregates
- Both Abcf2 and a Abcf3 (Gcn20), contribute to the processing of ordered (amyloid) aggregates.
- Abcf proteins are essential for animal development. De novo synthesis of *ABCF2* is required for gastrulation, nucleolar organization and embryonic development in *Xenopus*.
- Abcf2 knockdown results in aggregate-associated phenotypes in *C. elegans*



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References:
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