SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Oocyte Handling and Injections

Ovaries were surgically removed from adult female *Xenopus laevis* frogs that had been euthanized for 30 min in 0.05 % Benzocaine in water. Oocytes were treated with 2 mg/mL collagenase 1A (Sigma) in MMR by gentle rocking, until most of the oocytes were clearly dissociated. After several washes in MMR, stage I oocytes were separated from the rest using a 250 μm polypropylene mesh (Spectrum labs), and incubated for at least 6 hours in Oocyte Culture Medium (OCM) (Mir and Heasman, 2009). Oocytes were later injected with mRNAs encoding for indicated proteins by using a FemtoJet express microinjector (Eppendorf). Eppendorf Femtotips-1 were used for injections with settings according to manufacturer's instructions so that 100 picoliter would be delivered for each injection.

DNA and mRNA constructs

First strand cDNA was synthesized using total RNA (Trizol) isolated from stage I and stage 6 *Xenopus* oocytes using cDNA iScript Synthesis Kit (Bio-Rad). Xvelo, hnRNPA1, Tia1, Dazap1,Hbg1, Hba1, Caprin2 and Xcat-2 were PCR amplified from these cDNAs. FUS and CPEB3 ORFs were amplified from synthesized plasmids (gift from Avinash Patel and Jie Wang, Hyman Lab). First strand cDNA was also constructed from zebrafish newly laid eggs, and the ORF of bucky ball was PCR amplified from that library. Sequences of interest were cloned into a C-terminal pCS2-EGFP vector, an N-terminal EGFP-pCS2 vector (Caprin2, Dazap1 and Tia1) or a C-terminal pCS2-mCherry vector, all generated in this study, and sequenced. Xcat-2 and its 3'UTR, and mCherry coding sequences were cloned into pCS2 vectors. All vectors were linearized by NotI digestion prior to use of an SP6 based capped mRNA *in vitro* transcription kit (mMessage mMachine, Ambion) for coding mRNAs and MEGAscript SP6 kit (Ambion) for generating Xcat-2 and mCherry RNAs. ChromaTide Alexa Fluor 546-14-UTP was used to label Xcat-2 and mCherry RNAs.

Dividing Xvelo into Fragments

We divided the protein into pieces larger than 150 amino acid fragments – we believe smaller fragments would not be informative due to the disordered nature of the protein. In the alignments, conserved regions of protein sequence are scattered through the unconserved regions (Data S1A). We divided the protein into regions based on a multiple sequence alignment, which show changes in sequence conservation at different positions in the protein. For instance, the first 150 amino acids of Xvelo are very well conserved followed by an unconserved downstream region (around consensus 160). We picked this area for the start of fragment 2. After an immediate conserved region, there is a long unconserved patch of the protein that lasts until consensus residue 430 – this zone marks the end of Fragment 2. Fragment 3 is marked by a sequence of conserved, negatively charged residues (at around consensus 590 to 620). The sequence following the negatively charged patch was defined as fragment 4.

Immunoblot Analysis

After SDS Gel electrophoresis, the proteins were transferred to nitrocellulose membranes via wet transfer at 100 V for 2 hours. α-Xvelo was used at 1:2000. Goat anti-Rabbit IgG (H&L) Secondary Antibody DyLight 800 4X PEG conjugate (Thermo Scientific) was used at 1:15000. α-amyloid fibril OC (Millipore) was used at 1:1000. The blots were imaged by an Oddysey Infra-Red Imager (LICOR).

Xvelo Protein Purification from Insect Cells

Recombinant MBP-Xvelo-GFP, MBP-F1-WT-GFP, MBP-Xvelo-4D-GFP and MBP-Xvelo-8D-GFP, their RFP tagged versions, and their versions with no-fluorescent tag (for negative stain EM studies) were expressed in Sf9 insect cells using the baculovirus expression system. Briefly, insect cells were harvested in lysis buffer (50mM HEPES pH 7.6, 100 mM KCl, 1 M Arginine) in the presence of protease inhibitors and benzonase and homogenized by passing twice on Emulsifex on ice. The Arginine concentration was lowered down to 0.3 M before proceeding to column binding. The MBP (Maltose Binding Protein) was captured using Dextrin Sepharose Resin. Histidine (His) and MBP tags were cleaved off using a His-tagged Prescission Protease (MPI-CBG, in house) by incubation overnight on ice. Size exclusion chromatography was performed with a Superdex 200 16/60 HiLoad column using an Akta Pure fast protein liquid chromatography (FPLC) system (GE Healthcare Life Sciences).

Transmission Electron Microscopy

Balbiani bodies were dissected from stage I oocytes in OCM with 2% glutaraldehyde and fixed for two hours, then transferred to 0.5% glutaraldehyde and fixed overnight at 4^0 C. Samples were rinsed with OCM, then 0.1M cacodylate buffer pH 7.4, followed by post-fixation in 1% osmium with 0.8% potassium ferricyanide in cacodylate buffer for 1 hour on ice in a fume hood. Samples were rinsed in distilled water prior to staining in 1% aqueous uranyl acetate overnight at 4^0 C in the dark. The following day the samples were rinsed in distilled water, and dehydrated in an ethanol series using the progressive lowering of temperature method allowing 10 minutes at each step. They were brought to room temperature, rinsed in propylene oxide and infiltrated with 1:2, 2:1 solutions of propylene oxide:epon araldite 30 minutes each. Samples were then rinsed in 100% epon araldite for 2 hours before embedding and polymerizing at 65^0 C for 48 hours. Thin sections (75 nM) were made using a Reichert Ultracut S ultramicrotome, collected on EM slot grids, stained with 1% uranyl acetate in 50% methanol followed by 1% lead citrate. Samples were viewed on a Technai G^2 Spirit BioTWIN microscope and imaged with an AMT 2k CCD camera.

For negative staining, the self-assembly reaction for Xvelo-WT and Xvelo-4D proteins were initiated *in vitro* by lowering arginine concentration to 30 mM. 4 µl of the resulting protein solution was deposited on a formvar-coated mesh EM grid and stained for 30 s with 4 µl of a 1 % (w/v) uranyl formate solution. Excess stain was removed and the grids air-dried. Imaging was performed with a Tecnai 12 Biotwin TEM (FEI, The Netherlands) at 100 kV with a TVIPS 2k CCD camera (TVIPS GmbH, Gauting, Germany).

Thioflavin T binding

The ThT binding protocol was adapted from Alberti et al., 2009. Protein concentrations were determined by measuring absorption at 280 nm using calculated extinction coefficients, and by the SDS-PAGE band intensity compared with BSA standards. The concentrations were adjusted such that the protein concentration before arginine dilution was 15 µM and after dilution was 1.5 µM. The proteins were kept in a high arginine buffer (50 mM HEPES pH 7.6, 100 mM KCl, 300 mM Arginine), and diluted 10 fold into the self-assembly buffer (50 mM HEPES pH 7.6, 100 mM KCl) to start the self-assembly reaction. Self-assembly reactions were performed in black nonbinding microplates (Corning, NY) with 200 µl per well with agitation at 25°C. Samples were taken at indicated time points, and Thioflavin T was added to a final concentration of 1.5 µg/ml. Fluorescent plate reader was set at 450 nm excitation, 482 nm emission. Mot3 negative control was processed as described in Alberti et al., 2009 but at the final stage, resuspended in the self-assembly buffer with 30mM Arginine.

Mass Spectrometry

Freshly dissected Balbiani bodies from stage I oocytes and whole stage I oocytes were dissolved in 6 M Guanidine hydrochloride (GuaCl). The eggs were processed separately according to (Wühr et al., 2014) because of their high yolk content. Sample concentrations were adjusted after micro-BCA measurements so that a final protein concentration of 1 µg protein was present in each sample. Samples were diluted with 10 mM EPPS pH 8.5 to 2 M GuaHCl and a minimum of 10 ng/ ml LysC was added to the tubes. After overnight incubation at room temperature, the samples were further diluted to 0.5 M GuaCl, and a minimal amount of 10 ng/ ml LysC and 5 ng/ ml Trypsin were added. After an 8 hour incubation at 37 °C, samples were speed-vacuumed, and the resulting pellet was resuspended in 200 mM EPPS pH 8.0. 4 µl of TMT stock solutions (0.2 mg/40 ml in acetonitrile) were added to 40 µl of samples, and samples were incubated 2 hours at room temperature. TMT was quenched with 0.5 % final concentration of hydroxylamine. The samples were combined in one tube, acidified by 5 % phosphoric acid, and subjected to C18 solid-phase extraction (SPE) (SepPak, Waters) to desalt and isolate peptides. Resulting peptides were analyzed by an Thermo Fisher Orbitrap Fusion using the MultiNotch MS3 Method (McAlister et al., 2014).

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