Supplementary Information

Nuclear import of an intact preassembled proteasome particle

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The Supplementary Information includes the following material:

- Supplemental Tables S1, S2 and S3.
- Supplemental Figures S1 and S2.
- Supplemental Methods.
- Supplemental References.

Supplemental Table S1. Proteasome subunits identified by in solution mass spectrometry of isolated 26S particles from egg cytosol. A sample of the 26S particle preparation was subjected to tryptic digestion in solution, followed by analysis on an ion-trap mass spectrometer. All the known *bona fide* proteasome subunits are abundantly present in this sample and characterized by high spectral counts. Detailed analysis of all three types of proteasome particles and a comparison to proteasomes isolated from other systems, will be presented elsewhere.

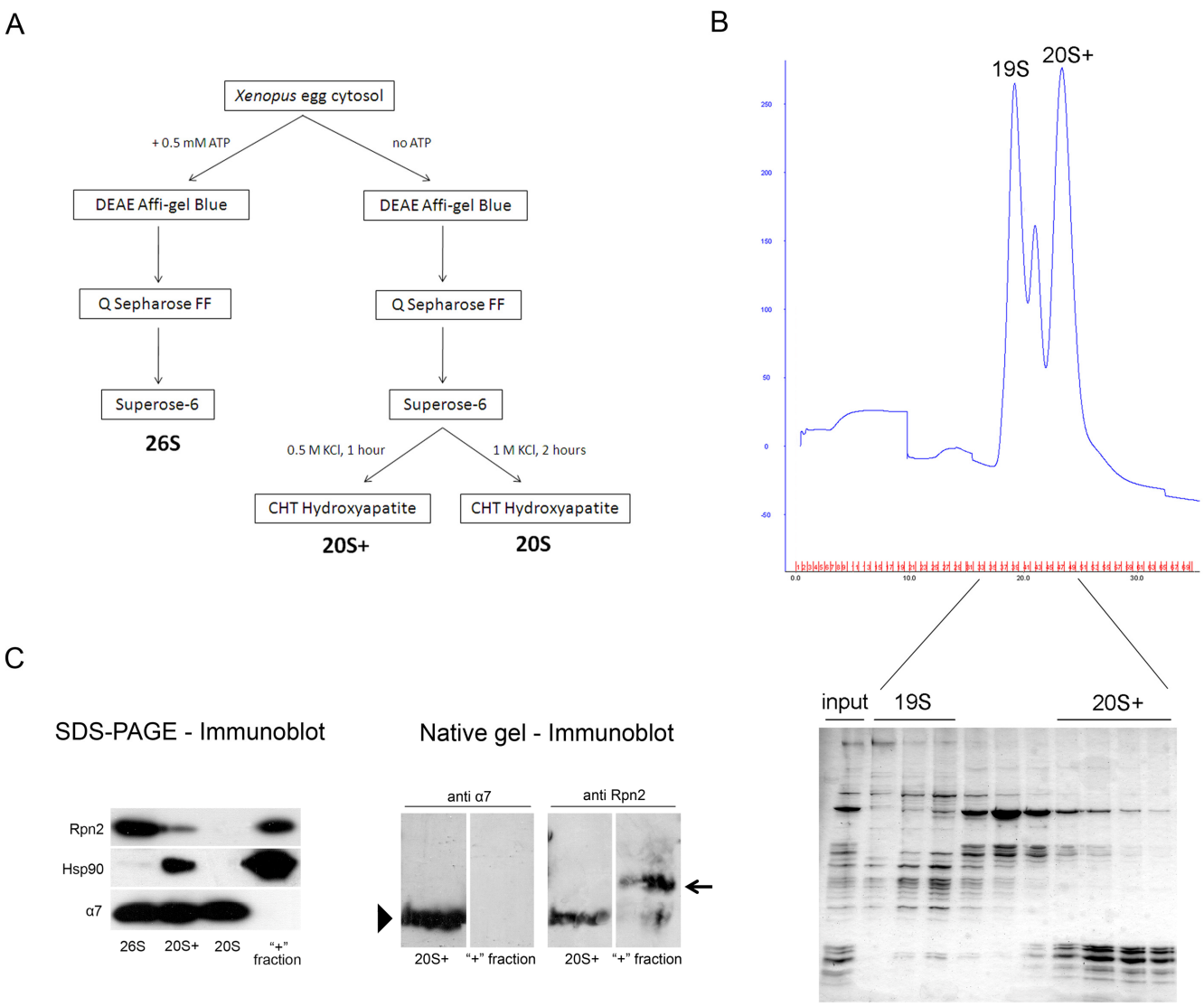
GI	Uniprot Accession	Protein name	PP Probability	Percent Coverage	Unique Peptides	Spectral Counts
148228229	Q58E21	PSMA1 (α6)	1	80.8	39	52
130852	P24495	PSMA2 (α2)	1	87.2	51	70
27371303	Q8AVD2	PSMA3 (α7)	1	56.9	23	38
76779672	Q3KPN6	PSMA4 (α3)	1	80.1	40	51
51968292	Q68A89	PSMA5 (α5)	1	62.7	23	40
50603942	Q6AZR7	PSMA6 (α1)	1	72	33	47
49522772	Q9PVQ1	PSMA7b (α4)	1	69.2	39	54
114107802	Q7ZYL1	PSMB1 (β6)	1	77	35	49
77748113	Q6GQ40	PSMB2 (β4)	1	79.9	35	49
56270462	Q5PQ24	PSMB3 (β3)	1	54.6	22	30
33417170	Q7T0M9	PSMB4 (β7)	1	50.8	31	40
148228778	Q3KPV9	PSMB6 (β1)	1	76.5	21	33
147906595	Q68EX4	PSMB7 (β2)	1	55.6	26	33
161611764	A9JS79	PSMB8 (β5)	1	59.3	28	35
32484378	Q7SYS3	PSMC1 (Rpt2)	1	68.6	42	51
163916327	A9ULV8	PSMC2 (Rpt1)	1	75.8	57	76
32450569	Q7SZ30	PSMC3 (Rpt5)	1	66.7	41	54
37994750	Q6PAD3	PSMC4 (Rpt3)	1	69.8	46	55
49255977	Q6GQB7	PSMC5 (Rpt6)	1	71.6	52	70
28278099	Q7ZX98	PSMC6 (Rpt4)	1	68.9	37	45
147905374	Q498L1	PSMD1 (Rpn2)	1	56.4	26	29
46249663	Q6NTK4	PSMD2 (Rpn1)	1	63.8	72	86
148228892	Q6GNC1	PSMD3 (Rpn3)	1	52.6	41	55
148235905	Q4V845	PSMD4 (Rpn10)	0.8748	55.3	3	3
56789608	Q5M7C5	PSMD5 (S5b)	1	43.4	17	17
54038456	Q5XGT1	PSMD6 (Rpn7)	1	66.3	43	49
147906376	Q66IY6	PSMD7 (Rpn8)	1	69.1	36	40
124481860	A2RV94	PSMD8 (Rpn12)	1	65.3	6	6
146327494	A5D8M2	PSMD10 (Nas6/gankyrin)	1	61.3	10	11
148236500	Q6AXA3	PSMD12 (Rpn5)	1	62.1	34	40
148233662	Q66IX2	PSMD13 (Rpn9)	1	85.7	48	59
49258176	Q7ZX92	PSMD14 (Rpn11)	1	63.2	29	33
133777072	Q6GN67	ADRM1-A	1	29.7	10	10
76779916	Q7ZXD6	ADRM1-B	1	35.9	11	11
46250075	Q6NUB1	PAAF1 (thul16) (Rpn14)	1	22	9	10
115292033	Q0D260	Ubiquitin carboxyl-terminal hydrolase UCHL5	1	67.2	19	21

Supplemental Table S2. Unique high molecular weight polypeptides identified in the 20S+ particle preparation. The primary proteins co-purifying with the 20S CP in the samples designated as "20S+" were excised from the ~80-110 kDa region of denaturing gels and subjected to in gel tryptic digestion and mass spectrometry analysis. High coverage proteins that were identified in multiple preparations of the 20S+ particle are compiled in this table.

GI	Uniprot Accession	Description	Annotation
46249663	Q6NTK4	PSMD2 (Rpn1)	Proteasome
147905374	Q498L1	PSMD1 (Rpn2)	Proteasome
54873686	Q6AZV1	Hsp90 beta protein	Heat shock protein
58177833	Q5FWY4	DNA replication licensing factor mcm6	Replication licensing
83405215	Q2TAF3	Echinoderm microtubule-associated protein-like 4 (emap-4)	Microtubule stabilizing
52139137	Q640K1	Neurochondrin	Signal transduction
168693593	B0LM40	Importin beta	Nuclear transport

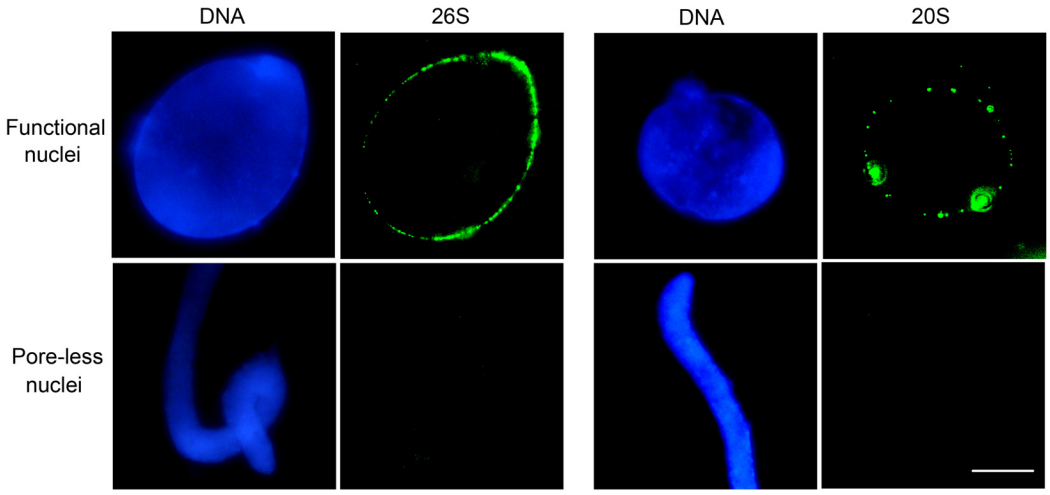
Supplemental Table S3. Major components of the isolated “+” fraction. Proteins dissociated from the proteasome core particle were further purified by gel filtration chromatography (see: Materials and Methods) and the isolated “+” fraction was functionally defined by the import assays shown in Figure 7. Three separate samples of the “+” fraction were analyzed by mass spectrometry. Only proteins that appeared in all three samples, with a Protein Prophet probability of 0.99-1 and that were identified by at least two unique peptides, are shown.

GI	Uniprot Accession	Description	Annotation
46249663	Q6NTK4	PSMD2 (Rpn1)	Proteasome
147905374	Q498L1	PSMD1 (Rpn2)	Proteasome
54873686	Q6AZV1	Hsp90 beta protein	Heat shock protein
108935850	P55861	DNA replication licensing factor mcm2	Replication licensing
82241532	Q7ZXB1	DNA replication licensing factor mcm7	Replication licensing
147900810	Q569Z1	Eukaryotic translation initiation factor 3 subunit B (eIF3b)	Translation
168693593	B0LM40	Importin beta	Nuclear transport



Supplemental Figure S1. Purification of active proteasome particles from *Xenopus* egg cytosol. (A). A flowchart summarizing the purification scheme for the three active proteasome particles, as detailed in Materials and Methods. ATP was included in all the buffers of the 26S purification protocol to preserve the structure of the proteasome holoenzyme. By contrast, ATP was omitted from the buffers used in 20S/20S+ purifications. Following Superose-6 gel filtration, separate protocols differentiated between 20S+ and 20S particles by the stringency of salt treatment followed by CHT Hydroxyapatite chromatography. (B) An example of 20S+ purification showing a typical elution profile from the last step of purification (CHT Hydroxyapatite chromatography). Protein content was visualized by silver staining. Only fractions labeled "20S+" showed peptidase activity, which marks the presence of proteasome core particles. (C) Comparison of the three proteasome particle preparations with the purified "+" fraction, which contains proteins that dissociate from proteasomes fractionated over a CHT

Hydroxyapatite column. A denaturing SDS-PAGE immunoblot (left) shows that Rpn2 and Hsp90 are present in the "+" fraction, while α7 is missing from it. A non-denaturing immunoblot (right) demonstrates that Rpn2 and α7 co-migrate in the 20S+ particle (arrowhead; see also Figure 3C), while the dissociated Rpn2 in the "+" fraction migrates at a different position (thin arrow). Note that proteins migrate in native gels as a complex function of molecular mass and exposed surface charge.



Supplemental Figure S2. Nuclear rim staining by 26S and 20S particles requires NPCs. Fluorescently labeled 26S and 20S particles were added into normal reconstitution reactions (Functional nuclei), or reactions in which NPC assembly was inhibited by BAPTA (Pore-less nuclei). Reactions were further incubated for 30 min and samples were fixed and analyzed by epifluorescence microscopy. Both the 26S and 20S particles accumulated at the nuclear envelope of functional nuclei, producing a punctuate rim staining. No staining was observed in BAPTA-inhibited intermediates, which contain fully sealed nuclear membranes with no nuclear pores. Scale bar, 10 μm.

SUPPLEMENTAL METHODS

In solution digestion

The proteins of the 26S preparation were denatured by the addition of 8M Urea, reduced with 10 mM DTT at 60°C for 30 min, modified with 100 mM iodoacetamide in 10 mM ammonium bicarbonate and trypsinized in 10 mM ammonium bicarbonate containing modified trypsin (Promega) at a 1:50 enzyme-to-substrate ratio, overnight at 37°C.

In gel digestion

The proteins of the 20S+ preparation were resolved by SDS-PAGE and silver stained. The protein bands of the ~80-110 kDa region were excised, reduced with 10 mM DTT, modified with 40 mM iodoacetamide and trypsinized at a 1:100 enzyme-to-substrate ratio.

Mass spectrometry analysis

The resulting tryptic peptides were resolved by reverse-phase chromatography on 0.075 X 200-mm fused silica capillaries packed with Reprosil reversed phase material (Dr. Maisch GmbH). The peptides were eluted with linear 65 minute gradients of 5 to 45% and 15 minutes at 95% acetonitrile, with 0.1% formic acid in water, at flow rates of 0.25 µl/min. Mass spectrometry was performed by an ion-trap mass spectrometer (Orbitrap, Thermo) in a positive mode using repetitively full MS scan followed by collision induced dissociation (CID) of the 7 most dominant ions selected from the first MS scan.

Database search

The mass spectrometry data was analyzed using the Trans Proteomic Pipeline (TPP) Version 4.3 (Keller *et al.*, 2005). TPP-processed centroid fragment peak lists in mzXML format were searched against Xenbase (Bowes *et al*., 2010). Duplicate identical sequences were removed and the resulting 16930 predicted proteins were supplemented with their 16930 corresponding decoy sequences (as described in http://www.matrixscience.com/help/decoy_help.html). The database searches were performed using X! Tandem with k-score plugin through the TPP. Search parameters included: trypsin cleavage specificity with two missed cleavages, cysteine carbamidomethyl as a fixed modification, methionine oxidation and protein N-terminal acetylation as variable modifications. Peptide tolerance and MS/MS tolerance were set at 10 ppm and 0.8 Da, respectively. X! Tandem refinement included: semi style cleavage. Peptide and protein lists were generated following Peptide Prophet and Protein Prophet analysis using protein FDR of <1%.

Supplemental References

Bowes, J.B., Snyder, K.A., Segerdell, E., Jarabek, C.J., Azam, K., Zorn, A.M., and Vize, P.D. (2010). Xenbase: gene expression and improved integration. Nucleic Acids Res. D607-612.

Keller, A., Eng, J., Zhang, N., Li, X.J., and Aebersold, R. (2005). A uniform proteomics MS/MS analysis platform utilizing open XML file formats. Mol. Syst. Biol. *1*, 2005.0017.