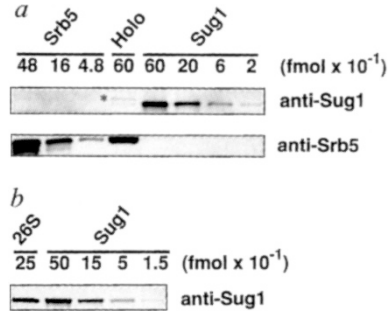
FIG. 3 Immunoblot analysis of Sug1 levels in *a*, RNA polymerase II holoenzyme (Holo), and *b*, the 26S proteasome. Sug1 and Srb5 standards are purified recombinant proteins. Srb5 is a stoichiometric subunit of holoenzyme[16]. The asterisk marks the principal immunoreactive band in the holoenzyme sample, which does not comigrate with recombinant Sug1.
METHODS. The 26S proteasome sample corresponds to fractions 21 and 22 of Fig. 1*d*–*f*. Holoenzyme was purified and characterized as described[16]. A number of proteins in addition to Srb5 are present at one copy per holoenzyme complex in this specific preparation[16]. The molar concentration of 26S proteasome was estimated from the total protein concentration, assuming 100% purity and M_r 2×10^6 (ref. 6). Protein concentrations were measured using the Protein Assay reagent (Bio-Rad) with BSA as standard.



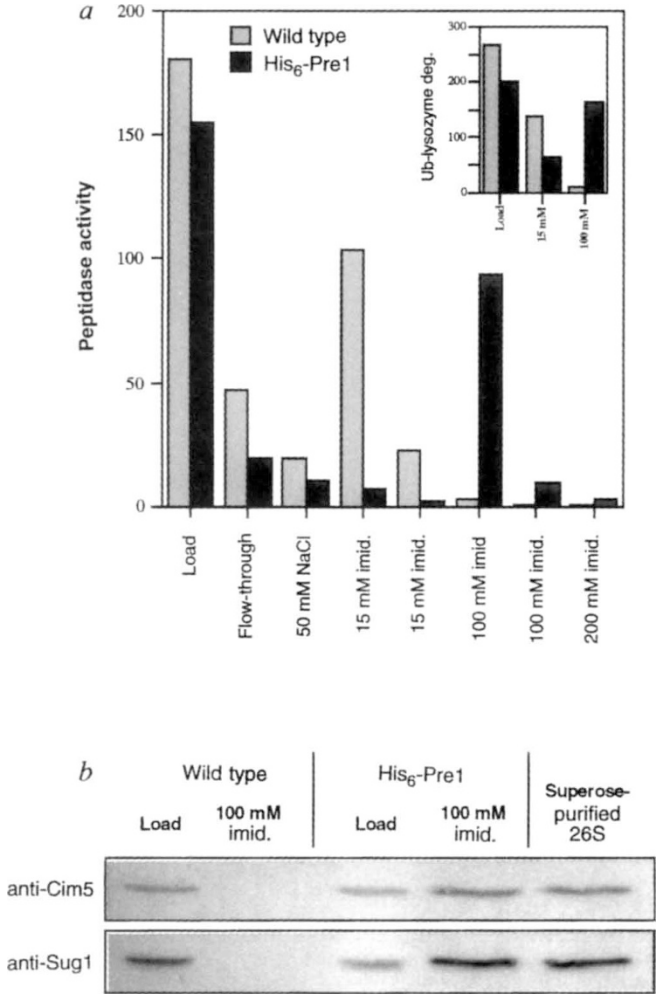


FIG. 2 Association of Sug1 with Pre1 shown by affinity chromatography. The Pre1 subunit of the 26S proteasome was tagged with a His_6 epitope. The His_6-Pre1 construct fully complemented a lethal *pre1* null mutation. Extracts from His_6-Pre1 and control strains were fractionated on Ni affinity columns. *a*, The epitope-tagged complex elutes at 100 mM imidazole (imid.), as indicated by either peptidase activity or (inset) ubiquitin-conjugate-degrading activity. Material was eluted from the column using a series of buffers (left to right). Activity values represent total activity in the fraction. *b*, Immunoblot of column fractions. Sug1 and Cim5 are detected in the 100 mM imidazole eluate of His_6-Pre1 but not wild-type extracts.
METHODS. Wild-type extract was from strain DY85; the His_6-Pre1 extract from SUB459, which is isogenic with YW071 (ref. 28) apart from the His_6 extension at the C terminus of Pre1. Extracts were processed as for Fig. 1 except that EDTA and DTT were omitted and Tris was replaced with sodium phosphate (50 mM) in all solutions. Eluates from the DEAE–Affigel blue column were loaded directly onto Ni–NTA (nitrilotriacetic acid) agarose columns. Nonspecifically bound proteins were eluted with a solution containing 50 mM NaCl, followed by a solution containing 15 mM imidazole and 100 mM NaCl. Equal proportions of the 100 mM imidazole eluate were immunoblotted for wild-type and 6HIS-Pre1 samples. Bands in the affinity eluate are more intense than in the load because a higher proportion of the eluate was immunoblotted.

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ERRATUM

Syk tyrosine kinase required for mouse viability and B-cell development

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In the legend to Fig. 4 of this Letter, the designations for rf2 and rf3 were confused: bold hatching represents rf2 and light hatching rf3. □