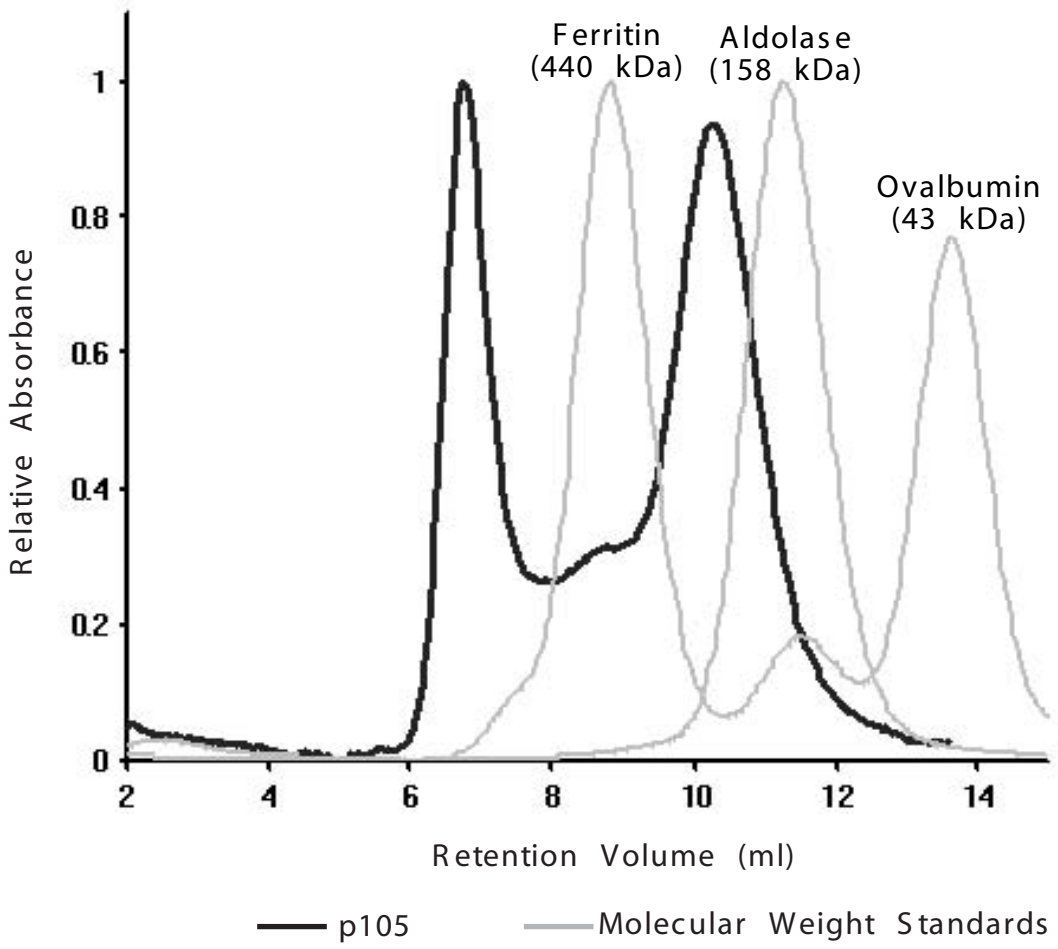

Supplementary Figure 1 : Gel filtration profile of recombinant p105 purified by refolding. The protein comes out in two separate peaks, the first corresponding to the excluded, aggregated fraction and the second corresponding to the folded protein. Only the folded fraction was used in our assays.
Note that comparison with various molecular weight standards shows that p105 exists as a dimer.

Protein Identification Summary

Mascot Search Results

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User            : chuan
Email           :
Search title    : 55 2955
MS data file    : D:\Data\022305\2955.D\2955.mgf
Database        : NCBInr 20050218 (2329789 sequences; 790010076 residues)
Taxonomy        : Homo sapiens (human) (129728 sequences)
Timestamp       : 24 Feb 2005 at 19:14:36 GMT
Significant hits: gi|55666609  PREDICTED: similar to Proteasome subunit beta type 1 (Proteasome component C5) (Mac
                  gi|51094758  proteasome (prosome, macropain) subunit, alpha type, 2 [Homo sapiens]
                  gi|4506201   proteasome beta 5 subunit [Homo sapiens]
                  gi|22538465  proteasome beta 3 subunit [Homo sapiens]
                  gi|55640605  PREDICTED: similar to Proteasome subunit alpha type 6 (Proteasome iota chain) (Macr
                  gi|565647    proteasome subunit HsC10-II [Homo sapiens]
                  gi|7106387   proteasome (prosome, macropain) subunit, alpha type 5 [Mus musculus]
                  gi|30582673  proteasome (prosome, macropain) subunit, beta type, 4 [Homo sapiens]
                  gi|4092058   proteasome subunit HSPC [Homo sapiens]
                  gi|32879911  proteasome (prosome, macropain) subunit, alpha type, 4 [Homo sapiens]
                  gi|4506179   proteasome alpha 1 subunit isoform 2 [Homo sapiens]
                  gi|20810439  Proteasome alpha 3 subunit, isoform 1 [Homo sapiens]
                  gi|4506195   proteasome beta 2 subunit [Homo sapiens]
                  gi|88166     C 3.4.25.1 proteasome endopeptidase complex () delta chain - human (fragment)
                  gi|4506203   proteasome beta 7 subunit proprotein [Homo sapiens]
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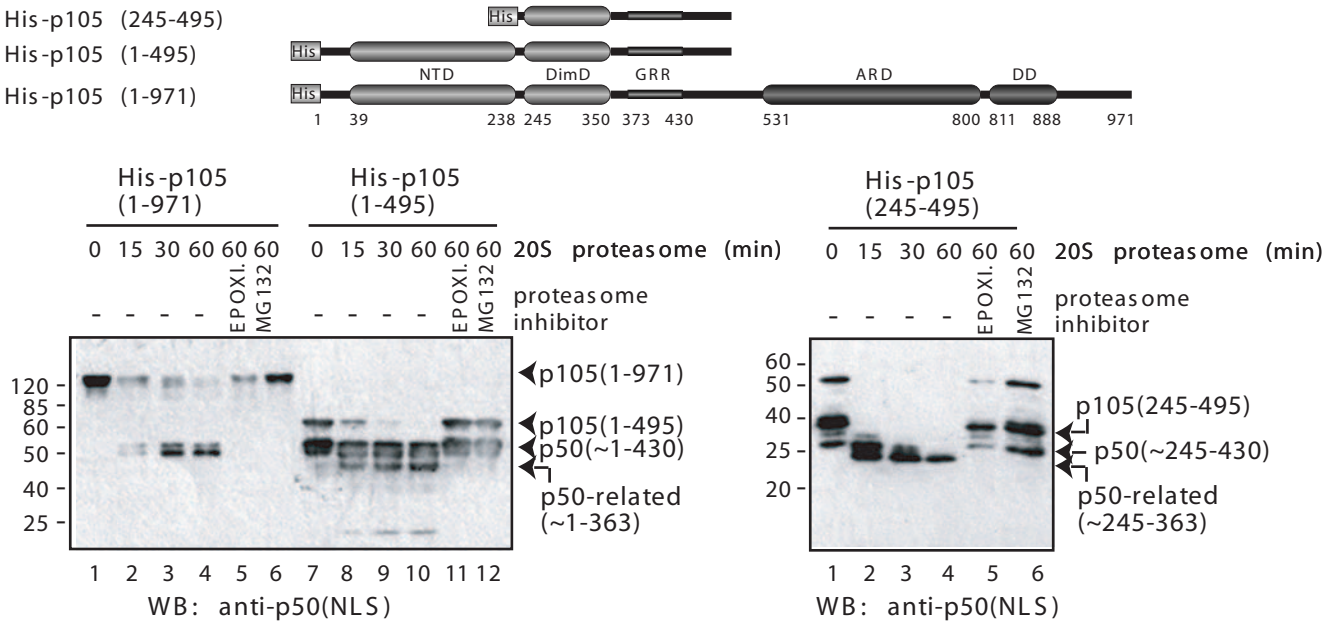
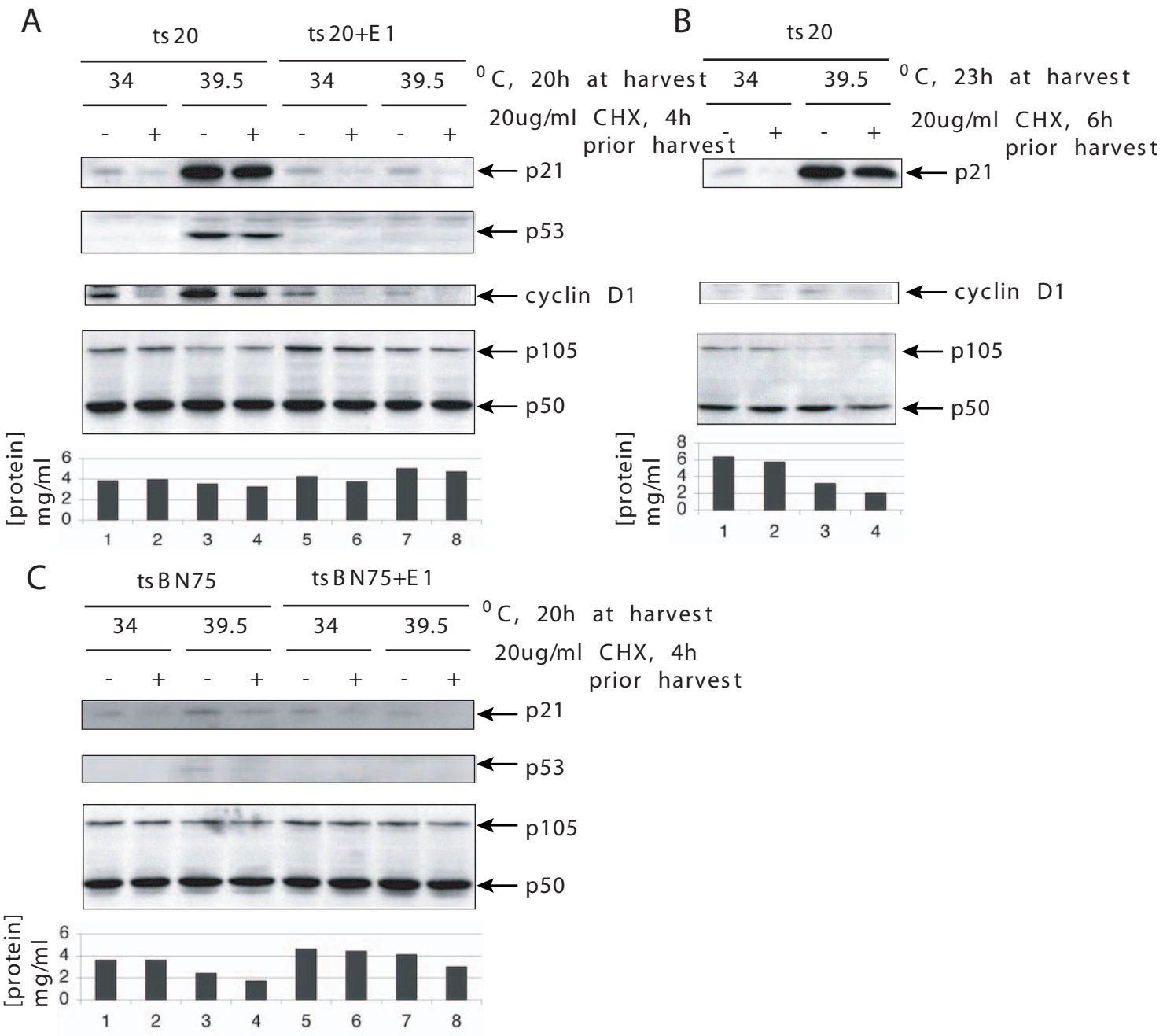
Supplementary Figure 2: Human 20S proteasome sample was reduced with DTT (10 mM), alkylated with iodoacetamide (55 mM), and digested with trypsin overnight before being analyzed by nano-LC-MS/MS. A search against the human sequences contained in the NCBInr database was performed and multiple proteasome proteins were identified. All proteins were identified with >95% confidence level. The top protein scores were very high and sequence coverage for proteasome subunit beta type 1 and alpha type 2 are 51% and 35%, respectively. For a complete listing of proteins identified please refer to the link provided below.

http://protid.scripps.edu/mascot/cgi/master_results.pl?file=..%2Fdata%2F20050224%2FF0577



Supplementary Figure 3: 20S proteasome generates aberrant p50-related products when C-terminally truncated p105 recombinant proteins were subjected to proteasomal degradation in vitro (left panel, lanes 8-10 and right panel, lanes 2-4). Only p50 from the full length p105 precursor is generated(left panel, lanes 2-4). The proteins were visualized by Western blotting with p50(NLS) antibody. Proteasome inhibitors epoxomicin and MG132 were used to ensure the reaction specificity.



Supplementary Figure 4: Temperature sensitive E1 mutant cell lines ts20TG<R> cells (Chowdary et al.,1994), tsBN75 (Nishitani et al.,1992) and corresponding E1 reconstituted cell lines ts20TG+E1 and tsBN75+E1 were obtained from Dr. James M. Roberts laboratory where these cell lines were recently used to show that CDK inhibitor p21<Cip1> degradation is independent of ubiquitination (Chen et al., 2004). Cells were seeded at equal density in 35mm plates and grown at the permissive temperature (34C) to 80% confluence, then cultures were shifted to the restrictive temperature (39.5C) or remained at the permissive temperature for the duration of the experiment as indicated (20h in panels A&C or 23h in panel B). Subset of cultures were treated with 20ug/ml cycloheximide for 4h or 6h prior to harvesting (panels A&C or panel B, accordingly). After incubation at different temperatures cells were rinsed with PBS and harvested in 50ul of lysis buffer containing 1%Triton X-100 and protease inhibitors cocktail. Total protein concentration in the lysates was determined with Bradford assay and is shown as a bar graphs corresponding to the conditions indicated for each sub-figure (A-C). Total protein concentration was indicative of how well cell survive different treatments as we noted that in some conditions, e.g. combination of ubiquitylation deficiency at non-permissive temperature and cycloheximide treatment, there was a loss of cell viability observed as the detachment of cells from culture plates; such cells were consequently lost to PBS wash at harvesting. 25ug of cellular protein was analyzed by Western blotting with p50NLS antibody (#1157, gift of Dr. Nancy Rice); cyclin D1(M20), p53(Pab 240), and p21(M19) antibodies from Santa Cruz. Results of Western are shown except where proteins were not detected in either cell conditions, e.g. cyclin D was not detected in tsBN75 or related tsBN75+E1 cells. The identity of p21 band was confirmed by two additional p21 antibodies, C19 and F5, both from Santa Cruz. Changes in p105/p50 ratio or reduction in the amount of either protein were not detected 4h or 6h after cycloheximide treatment in all cell lines and temperatures tested. Apparent toxicity of cycloheximide on ubiquitination-compromised background (at elevated temperatures in E1 mutant cells) precluded us to extend the treatment and address the question of ubiquitin-dependence of p105 processing in this experimental system.