s8a.m5.o3 **Cutting complexity down to size: Structural and mutational studies of the eukaryotic 20S proteasome.** M. Groll[1], R. Huber[1], M. Glickman[2], C. Crews[3], G. Bourenkow[4], H. Bartunik[4] and D. Finley[2]. *[1] Max-Planck-Institute für Biochemie, Martinsried, Germany. [2] Dept. of Cell Biology, Harvard Medical School, Boston, MA, USA. [3] Department of Molecular & Cellular Biology, Yale University, New Haven, CT. 06511, USA. [4] AG Proteindynamik MPG-ASMB c/o DESY, D-22603, Hamburg, Germany.*
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The proteasome is formed by the association of its core particle (CP) with the 19S regulatory particle (RP), which selects ubiquitin-conjugates for degradation. The RP is composed of the 8-subunit lid and 8-subunit base assemblies, the base lying proximal to the CP. The proteolytic active sites of the proteasome are contained within the luminal space of the CP, suggesting a model in which substrates are fully unfolded before being translocated through a channel into the CP lumen.

The crystal structure of the free CP from yeast shows no evidence of a channel through which substrates might gain access to the hydrolytic lumen. Free CP has little activity against peptides, but is stimulated in the presence of either the base or the complete RP. To test whether this "activation" might reflect opening of a channel as a consequence of binding of the base to the CP, we deleted CP subunit segments that we suspected to "plug" the hypothetical channel, as inferred from the crystal structure of the CP. Deletion of the N-terminus of the α3 subunit resulted in constitutive activation of the free CP for peptide hydrolysis. The mutant CP cannot be further activated by standard methods. It is noteworthy that the mutant has much higher basal activity than the wild type; this implies that "activation" reflects a derepression of the CP. The crystal structure of the CP from the α3 deletant revealed that the mutation resulted in the formation of an axial channel into the CP, but not in gross structural rearrangements. Importantly, the crystallographic data showed that activation was not the result of an allosteric alteration of the active site architecture. Rather, the effect of the deletion on peptide hydrolysis must be due to more rapid entry of peptides into the lumen. In summary, these data indicate that 1) there is a channel in the CP, allowing for substrate entry, 2) the channel was not seen by crystallography because it is gated; i.e., it is closed in the free form of the complex, 3) due to the closed structure of the channel of the free CP in solution, the rate of peptide hydrolysis by the free CP is limited by the entry of substrates into the chamber, 4) classical "activation" of the CP reflects opening of this channel, and 5) the channel is opened when the base contacts the CP, since the α3 deletion has little or no effect on peptide hydrolysis by the 26S proteasome holoenzyme. The chaperone site and substrate channel identified in the present studies presumably represent the final two stations for substrates as they migrate into the lumen of the CP for hydrolysis.

s8a.m5.o4 **Crystal structure of a gamma-herpesvirus cyclin/cdk complex.** G.L. Card, P. Knowles & N.Q. McDonald^ *Structural Biology Laboratories, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, Holborn, London, WC2A 3PX, UK and ^Department of Crystallography, Birkbeck College, Malet Street, WC1E 7HX, UK.*
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Several gamma-herpesviruses encode proteins related to the mammalian cyclins, regulatory subunits of cyclin-dependent kinases (cdk) essential for cell cycle progression. We have determined a 2.5 A crystal structure of a full length oncogenic gamma-herpesvirus cyclin complexed to cdk2. The viral cyclin binds cdk2 with a different orientation to cyclinA and makes several novel interactions at the interface, yet it activates cdk2 by triggering similar conformational changes as cyclinA. Residues in the cdk interface are conserved amongst the viral and the cellular D-type cyclins suggesting that this structure has wider implications for other cyclin/cdk complexes. The observed resistance of this viral cyclin/cdk complex to inhibition by the p27Kip cdk inhibitor is explained by sequence and conformational variation in the cyclin rendering the p27Kip binding site on the cyclin subunit non-functional.