Taylor & Francis
Taylor & Francis Group

Nucleus

ISSN: 1949-1034 (Print) 1949-1042 (Online) Journal homepage: https://www.tandfonline.com/loi/kncl20

Proteasomes crossing the nuclear border

Anca F. Savulescu, Asaf Rotem & Amnon Harel

To cite this article: Anca F. Savulescu, Asaf Rotem & Amnon Harel (2011) Proteasomes crossing the nuclear border, Nucleus, 2:4, 258-263, DOI: 10.4161/nucl.2.4.16267

To link to this article: https://doi.org/10.4161/nucl.2.4.16267

Copyright © 2011 Landes Bioscience

Published online: 01 Jul 2011.

Submit your article to this journal

Article views: 229

View related articles

Citing articles: 11 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=kncl20

Nucleus 2:4, 258-263; July/August 2011; © 2011 Landes Bioscience

Proteasomes crossing the nuclear border

Anca F. Savulescu, Asaf Rotem[†] and Amnon Harel*

Department of Biology; Technion-Israel Institute of Technology; Haifa, Israel

[†]Current address: Department of Biological Chemistry and Molecular Pharmacology; Harvard Medical School; Boston, MA USA

Keywords: nuclear pore complex, 26S proteasome, nuclear import, Ran GTPase, nuclear bodies.

Submitted: 05/02/11

Accepted: 05/16/11

http://dx.doi.org/10.4161/nucl.2.4.16267

*Correspondence to: Amnon Harel;
Email: amharel@tx.technion.ac.il

Extra View to: Savulescu AF, Shorer H, Kleifeld O, Cohen I, Gruber R, Glickman MH, et al. Nuclear import of an intact preassembled proteasome particle. Mol Biol Cell 2011; 22:880–91; PMID:21289101; http://dx.doi.org/10.1091/mbc.E10-07-0595

Proteasomes localize to different compartments in eukaryotic cells, but the details of this dynamic distribution are poorly understood. Here, we discuss evidence linking proteasome activity to specific intranuclear locations and the potential mechanisms that may target these large proteolytic machines to the nucleus. Recent work has demonstrated the targeting of intact proteasome particles to newly formed nuclear compartments in a cell-free reconstitution assay. We discuss the difference between open and closed mitosis systems and the targeting of proteasomes to the nuclear periphery, as well as the nucleoplasm. Finally, we present a potential model for regulated assembly of the 26S proteasome holoenzyme inside the nucleus.

The proteasome is a highly conserved supramolecular machine that degrades proteins in an ATP-dependent fashion in all eukaryotes.[1,2] The proteasome holoenzyme, or 26S particle, is composed of a central barrel-shaped core particle (the 20S CP), flanked by one or two regulatory complexes (19S RPs). Polyubiquitinated proteins are targeted to the 19S RP, unfolded and inserted into the internal chamber of the 20S CP, where they are cleaved into short peptides.[1,3,4] Proteasomes localize to different cellular compartments, including subnuclear domains, but the targeting mechanisms and the regulation of this complex distribution remain poorly understood.

Proteasome Localization and Subnuclear Domains

In rapidly growing yeast cells the majority of proteasomes have been reported to be localized inside the nucleus.[5-7] This is thought to be the result of nuclear import of newly synthesized proteasome subunits, which enter the nucleus as inactive precursor complexes.[8-10] In higher eukaryotes, different reports have associated proteasomes with nucleoli, euchromatin, the periphery of heterochromatin and with specific intranuclear subcompartments, or nuclear bodies, such as PML nuclear bodies.[7,11-16] The accumulation of proteasomes in ring-shaped nuclear bodies, termed clastosomes, occurs in response to stimuli that activate proteasome-dependent proteolysis and can be reversed by inhibiting this activity.[17] This suggests that at least some types of nuclear bodies may represent specialized intranuclear degradation centers.[18,19] In support of this view, nuclear bodies have been found to be enriched in specific populations of transcriptional regulators modified by ubiquitin or by the small ubiquitin-like modifier (SUMO).[17,20] A related hypothesis is that proteasomes that are recruited into nuclear bodies may have specific configurations, which are functionally distinct from those of proteasomes that are diffusely present throughout the nucleoplasm.[18]

Although different lines of evidence support the view that proteasome-mediated degradation of proteins can occur inside the nucleus, it is by no means clear if this is the general rule for nuclear substrates.[19,21,22] One established role of the ubiquitin-proteasome system within the nucleus is the rapid inactivation of regulatory factors, as exemplified by the yeast Matα2 and the human MyoD proteins.[23,24] Degradation of the tumor suppressor p53 was once considered to be exclusively cytoplasmic, but is now thought to occur in both the

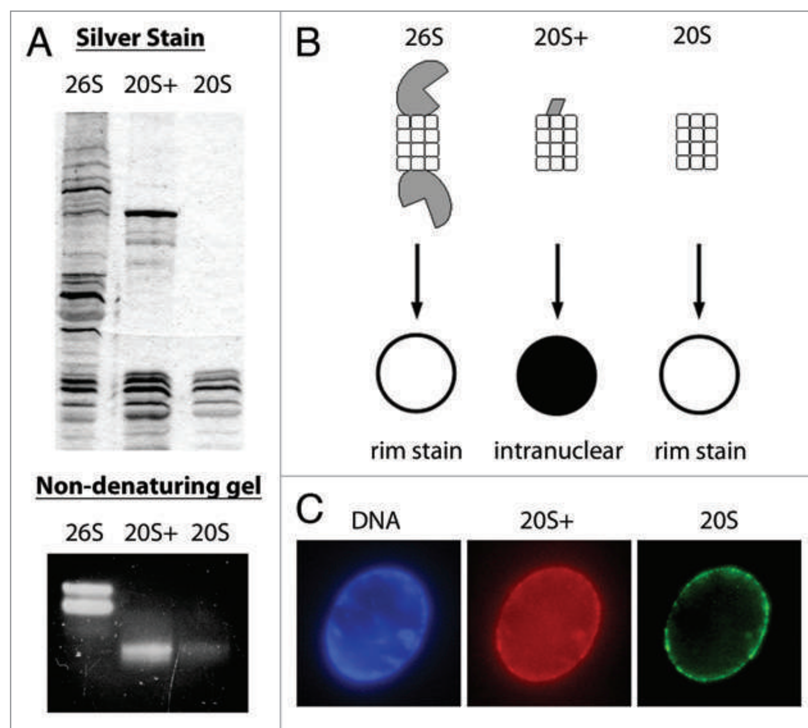

Figure 1. Nuclear targeting of intact proteasome particles in a cell-free system. (A) Silver staining pattern (top) and migration in a non-denaturing gel (bottom) of three types of active proteasome particles purified from Xenopus egg extract. The 26S holoenzyme and 20S CP are well known from other eukaryotic systems, while the 20S$^+$ particle is a novel intermediate form. Proteasomes in the non-denaturing gel were visualized by the fluorogenic peptide Suc-LLVY-AMC. (B) Schematic representation of the three proteasome particle types and the targeting pattern obtained with fluorescently labeled particles in the cell-free system. (C) Simultaneous detection of fluorescently labeled 20S$^+$ (red) and 20S CP (green) in the same nucleus. DNA was stained with Hoechst 33258. See reference 38 for further details.

cytoplasm and the nucleus.[21,25] The complex regulation of p53 activity involves nucleocytoplasmic shuttling, mono- and poly-ubiquitination and the integration of input from multiple signaling pathways.[26] Individual subunits and subcomplexes of the 19S RP have also been shown to have non-proteolytic roles inside the nucleus and to be recruited to specific gene promoters in various eukaryotes.[27-29]

The overall picture emerging from various studies and different organisms is of complex and dynamic localization of proteasomes within the nucleus. This includes dynamically assembled modification and/or degradation foci, sequestering of specific types of substrates (and perhaps also of proteasomes) and translocation of some substrates across the nuclear envelope.[16,18] Until recently, it has been assumed on the basis of the well-studied example of budding yeast, that active proteasomes are assembled inside the nucleus from separately imported precursors.[9,10] However, early work in mammalian cells has hinted at the possibility that intact proteasome particles may also be capable of crossing the nuclear border.[30,31]

The only known gateway for nucleocytoplasmic exchange is the nuclear pore complex (NPC), a huge multiprotein assembly embedded within the double membranes of the nuclear envelope. NPCs mediate bidirectional transport of a wide array of macromolecular cargoes, including some very large particles such as ribosomal subunits, mRNPs and certain types of viral capsids.[32-35] Importantly, in multicellular eukaryotes the whole nuclear envelope disassembles and reforms once in every cell cycle.[36] This creates another opportunity for nuclear targeting of whole proteasome particles or specific subunits, by a potential interaction with decondensing chromosomes at the exit from mitosis. By contrast, yeast and other unicellular eukaryotes are characterized by a closed mitosis and maintain an intact nuclear envelope throughout the cell cycle.[34,37] This leaves NPCs and nuclear import as the only possible means for intranuclear localization of proteasomes in unicellular eukaryotes.

Nuclear Targeting in a Cell-Free System

To study the nuclear targeting of proteasomes in vertebrates, we used a cell-free reconstitution system based on Xenopus egg extracts.[38] This in vitro system recapitulates the events that follow fertilization during normal development and the process of nuclear assembly at the exit from mitosis in vertebrate cells. The reconstitution assay is also amenable to biochemical manipulation and the use of specific inhibitors of nuclear assembly.[39-41] Using interphase egg extracts, we reconstituted nuclei with functional NPCs and followed the redistribution of intact proteasome particles between the nuclear and cytoplasmic compartments. We found three types of stable, proteolytically active proteasomes particles in egg cytosol and these showed differential targeting behavior towards newly formed nuclear compartments in the reconstitution system (summarized in **Fig. 1**).[38]

Proteasome particles were not targeted to chromatin at early stages of nuclear assembly and showed no affinity towards pore-free nuclear membranes. However, when mature, functional NPCs were present within the nuclear envelopes, two types of proteasome particles were able to localize to the nuclear periphery. These two forms, the 20S CP and the 26S holoenzyme, were targeted to the nuclear envelope but did not reach the nuclear interior. A third, novel form of the proteasome, which we designated as the 20S$^+$ particle, was imported through NPCs and accumulated in the nucleoplasm (**Fig. 1**).

Biochemical purification of the three proteasome particles followed by mass spectrometry analysis provided some initial clues regarding their different targeting properties. The novel 20S$^+$ particle contained the 20S CP, two large 19S RP subunits and a few additional co-purifying proteins. These additional proteins

included the shuttling transport receptor importin β and the molecular chaperone Hsp90. An import-compatible form of the proteasome could be reconstituted from isolated 20S core particles and a subset of purified interaction partners. This suggests that the $20S^+$ particle represents an intermediate form, between the well-known 20S CP and 26S holoenzyme, which is recognized as a legitimate nuclear import cargo in this system derived from unfertilized amphibian eggs.

Surprisingly, $20S^+$ import was not blocked by intervention in the Ran GTPase cycle. Ran is a small GTPase which regulates most nuclear transport pathways by inducing cargo release in the target compartment.[42,43] Our results indicate that despite the presence of importin β, the targeting of the $20S^+$ proteasome particle to the nuclear interior does not occur through one of the canonical import pathways. Specifically, cargo release within the nucleoplasm is probably not triggered by RanGTP binding. It is interesting to note that Rpn1 and Rpn2, the two 19S RP subunits identified in the $20S^+$ particle, are structurally characterized by PC repeats.[44,45] These α-helical repeats resemble the HEAT repeats of many nuclear transport receptors of the importin β superfamily.[33,42] One hypothesis explaining the atypical import properties of $20S^+$ particles is that these PC repeats mimic the interactions within import receptor heterodimers. Alternatively, these proteins may be able to directly interact with the NPC, as has been suggested for another HEAT-repeat protein, β-catenin.[46]

A different question arising from our data concerns the functional role of the chaperone Hsp90, another unique component of the $20S^+$ proteasome particle. Hsp90 may be needed for blocking specific interaction surfaces on the proteasome core particle, thus halting the normal process of holoenzyme assembly and stabilizing an import-compatible particle form. Alternatively, Hsp90 may modulate the large proteasome particle during its passage through the NPC, or may even be involved in the termination of the import process inside the nucleus.

Further research on the specific components of the $20S^+$ proteasome and their interactions will be needed to reveal the unconventional pathway that leads this particle into the nucleus.

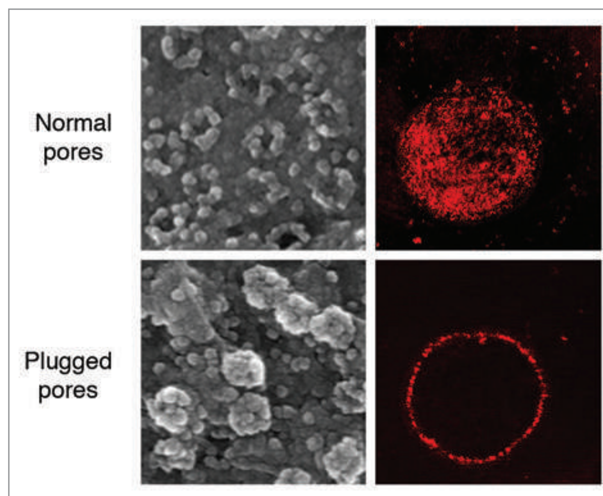

Figure 2. Proteasome docking can occur at the external surface of plugged NPCs. Nuclei were reconstituted in vitro and visualized by scanning electron microscopy (left), as in Rotem et al.[41] Recombinant Impβ 45-462 was used to plug pre-existing NPCs (bottom). TRITC-labeled $20S^+$ particles were added into reconstitution reactions after nuclear assembly (right). The labeled proteasomes accumulated inside control nuclei containing functional NPCs and showed a punctate nuclear rim staining when pores were plugged.

Docking on Nuclear Pores

As mentioned above, both the 26S holoenzyme and the 20S CP were targeted to the nuclear envelope in the cell-free reconstitution system.[38] The punctate nuclear rim staining pattern produced by these proteasome particles is suggestive of localization to NPCs. Indeed, when the known assembly inhibitor BAPTA was used to produce sealed, pore-free nuclear membranes, no proteasome targeting was observed. Therefore, both the nuclear import of $20S^+$ particles and the targeting of 26S and 20S particles to the nuclear periphery are NPC dependent.

Using another advantage of the in vitro assembly system, we assembled normal nuclei with functional NPCs and subsequently plugged these nuclear pores by adding the recombinant Impβ 45-462 fragment. Scanning electron microscopy demonstrates that the whole area of the central NPC channel is physically blocked by treatment with this truncated form of importin β (**Fig. 2**, bottom left). This confirms previous observations by atomic force microscopy and the notion that Impβ 45-462 irreversibly binds to multiple FG-repeat nucleoporins at the NPC.[47,48] When fluorescently labeled $20S^+$ particles were introduced to normal nuclei, they strongly accumulated in the nucleoplasm. However, when NPCs were first plugged by Impβ 45-462, the $20S^+$ particles produced a punctate nuclear rim staining pattern (**Fig. 2**, right; see also ref. 38).

Taken together, these results suggest that all three types of proteasome particles can dock onto normal NPCs, while only the $20S^+$ particle is able to proceed and translocate through the pore channel into the nucleoplasm. Docking can be hypothesized to occur through a common constituent of the three particle types (i.e., one or more of the core particle subunits). On the nuclear pore side, the prime suspect for mediating these putative interactions would be Nup358/RanBP2, a large multidomain nucleoporin which makes up the cytoplasmic filaments on the outer surface of the NPC.[49] As shown in **Figure 2**, access to most areas of the NPC is blocked by the treatment with Impβ 45-462, leaving only the outer extensions of the cytoplasmic surface as possible docking sites. This hypothesis can now be tested by immunodepleting Nup358/RanBP2 from egg extract, reconstituting cytoplasmic filament-free NPCs and testing proteasome targeting.

Intriguingly, Nup358/RanBP2 also provides a conceptual link to subnuclear

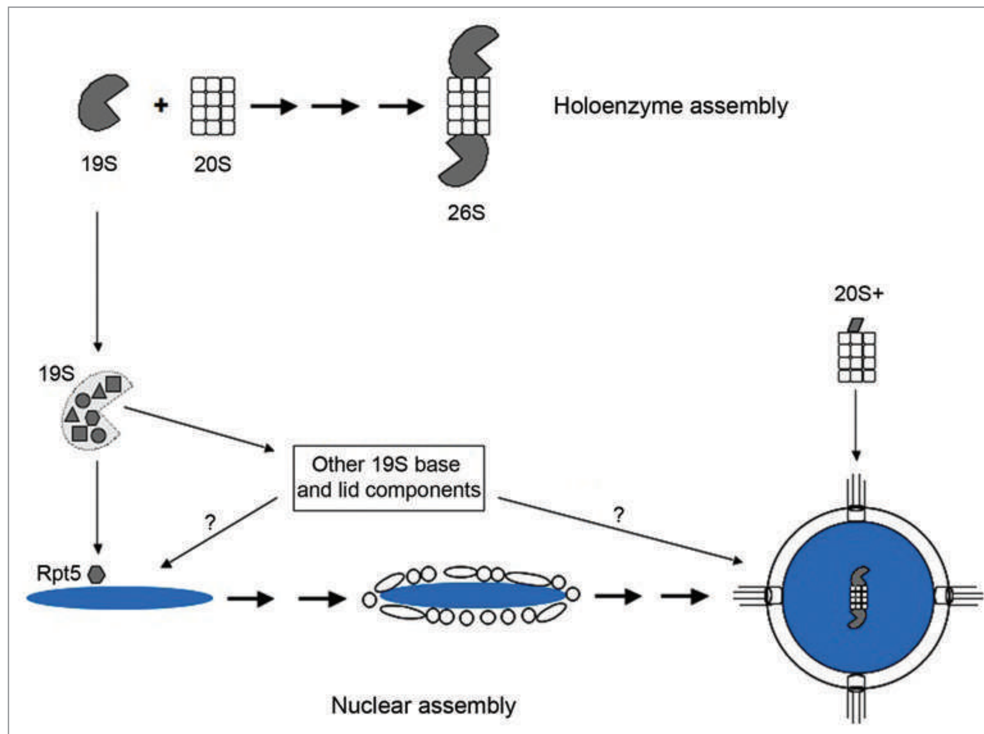

Figure 3. Potential model for 26S holoenzyme assembly inside the nucleus, following separate nuclear targeting pathways. Nuclear assembly is schematically depicted, starting from a condensed chromatin template (blue), proceeding through membrane vesicle binding and fusion and resulting in a complete nuclear envelope with functional NPCs. The Rpt5 subunit is targeted to early assembly intermediates, while the $20S^+$ particle (containing Rpn1 and Rpn2) is imported through mature NPCs. The fate of other 19S RP subunits remains to be investigated.

domains, which may serve as the ultimate destination for imported proteasome particles. As mentioned above, some types of nuclear bodies share a link to the ubiquitin-proteasome and SUMO-conjugation pathways.[18] Nup358/RanBP2 itself acts as a SUMO E3 ligase in vertebrates, resulting in the localization of RanGAP1 (and therefore GTP hydrolysis on Ran) to the outer cytoplasmic side of the NPC.[50] Our work provides the first clues for a possible dynamic mechanism targeting active proteasome particles to a very strategic position on the outer cytoplasmic surface of NPCs. It will be interesting to see if additional evidence for this type of targeting can be obtained from other eukaryotic systems.

The Fast Lane into the Nucleus

Our data points to one particular form of an intact preassembled proteasome, the $20S^+$ particle, as the molecular species which is recognized as a nuclear import cargo in the egg extract system.[38] This suggests a specialized mechanism allowing for the quick mobilization of intact particles into newly formed nuclei. The eggs of *Xenopus laevis* and other amphibians contain large stockpiles of materials, which are stored for use during the early developmental stages that follow fertilization. This is a static, arrested system, which is poised for the rapid changes triggered by fertilization.[51,52] Once activated, it relies on pre-existing protein complexes until the activation of the zygotic transcription program at the ~4,000 cell stage. It is therefore logical to assume that part of the existing reservoir of proteasome particles in eggs is destined for the nucleus and may be stored in an import-compatible form.

Could this type of nuclear targeting mechanism also work in other cellular scenarios? Actively growing yeast cells, which are characterized by a closed mitosis and a constant supply of newly synthesized proteasome subunits, represent an extremely different situation. Indeed, the current model for this scenario would be holoenzyme assembly inside the nucleus from independently imported and inactive precursors. Exciting new work from Eric Chang's laboratory suggests that even in unicellular eukaryotes a second, faster pathway into the nucleus may exist.[53] Working in the fission yeast, *Schizosaccharomyces pombe*, these authors have investigated Arc3-mediated mobility of proteasomes throughout the cell. Photobleaching experiments demonstrated that pre-existing proteasomes are rapidly imported into the nucleus, but exit it slowly. When Arc3 expression is silenced, proteasome import is substantially impaired and the cells become hypersensitive to DNA damage.[53] Thus, both in yeast and in vertebrates, rapid mobilization of intact proteasome particles may be needed for certain types of cellular response to changes in the environment. In the future, it will be interesting to see if proteasome import can be correlated to dynamic changes in specific intranuclear subcompartments, or orphan nuclear bodies.[18]

26S Assembly within the Nucleus

All three types of stable proteasome particles identified in Xenopus egg extract

are proteolytically active towards small peptides. However, in order to degrade polyubiquitinated proteins, the 19S RP would have to be complexed with the 20S CP. This suggests that the import of $20S^+$ particles observed in the cell-free reconstitution system may serve as a basis for the assembly of the full 26S holoenzyme inside the nucleus. The assembly mechanism of the 19S regulatory particle and 26S holoenzyme are still not fully elucidated and different assembly pathways, involving chaperones and accessory proteins, have been suggested.[54-57] Little attention has been given to the exact intracellular location of these assembly steps, especially in vertebrates. Since most of the 19S RP subunits are missing from the $20S^+$ particle, a putative mechanism for nuclear holoenzyme assembly would presumably involve the independent import of the remaining components.

Further work in the cell-free system revealed that at least one 19S subunit, which is missing from the $20S^+$ particle, is targeted to the nucleus by a separate mechanism. Rpt5, one of the six ATPases within the base subcomplex of the 19S RP, was detected inside BAPTA-inhibited assembly intermediates.[38] Since these nuclei are enclosed by sealed nuclear membranes with no NPCs, Rpt5 must be recruited early on, perhaps by interacting with chromatin. This leaves an open question regarding the fate of the remaining base and lid components of the 19S RP, but allows us to suggest a working model for proteasome holoenzyme assembly within the nucleus in organisms characterized by an open mitosis (**Fig. 3**). According to this hypothesis, the intact, preassembled $20S^+$ particle is imported through mature NPCs, while the remainder of the 19S RP subunits may be independently targeted to the nuclear compartment. An attractive feature of this model is that the final stages of 26S assembly can be regulated by intranuclear signals, such as DNA damage detection or cell cycle transitions, and may also be directed towards specific subnuclear locations.

Acknowledgments

This work was supported by grants from the Israel Science Foundation (813/05 and 1072/10) to A.H.

References

1. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 2002; 82:373-428.
2. Finley D. Recognition and processing of ubiquitin-protein conjugates by the proteasome. Annu Rev Biochem 2009; 78:477-513.
3. Glickman MH. Getting in and out of the proteasome. Semin Cell Dev Biol 2000; 11:149-58.
4. Pickart CM, Cohen RE. Proteasomes and their kin: proteases in the machine age. Nat Rev Mol Cell Biol 2004; 5:177-87.
5. Laporte D, Salin B, Daignan-Fornier B, Sagot I. Reversible cytoplasmic localization of the proteasome in quiescent yeast cells. J Cell Biol 2008; 181:737-45.
6. Russell SJ, Steger KA, Johnston SA. Subcellular localization, stoichiometry and protein levels of 26 S proteasome subunits in yeast. J Biol Chem 1999; 274:21943-52.
7. Wojcik C, DeMartino GN. Intracellular localization of proteasomes. Int J Biochem Cell Biol 2003; 35:579-89.
8. Isono E, Nishihara K, Saeki Y, Yashiroda H, Kamata N, Ge L, et al. The assembly pathway of the 19S regulatory particle of the yeast 26S proteasome. Mol Biol Cell 2007; 18:569-80.
9. Lehmann A, Janek K, Braun B, Kloetzel PM, Enenkel C. 20 S proteasomes are imported as precursor complexes into the nucleus of yeast. J Mol Biol 2002; 317:401-13.
10. Wendler P, Lehmann A, Janek K, Baumgart S, Enenkel C. The bipartite nuclear localization sequence of Rpn2 is required for nuclear import of proteasomal base complexes via karyopherin alpha-beta and proteasome functions. J Biol Chem 2004; 279:37751-62.
11. Adori C, Low P, Moszkovkin G, Bagdy G, Laszlo L, Kovacs GG. Subcellular distribution of components of the ubiquitin-proteasome system in non-diseased human and rat brain. J Histochem Cytochem 2006; 54:263-7.
12. Gordon C. The intracellular localization of the proteasome. Curr Top Microbiol Immunol 2002; 268:175-84.
13. Mattsson K, Pokrovskaja K, Kiss C, Klein G, Szekely L. Proteins associated with the promyelocytic leukemia gene product (PML)-containing nuclear body move to the nucleolus upon inhibition of proteasome-dependent protein degradation. Proc Natl Acad Sci USA 2001; 98:1012-7.
14. Palmer A, Rivett AJ, Thomson S, Hendil KB, Butcher GW, Fuertes G, et al. Subpopulations of proteasomes in rat liver nuclei, microsomes and cytosol. Biochem J 1996; 316:401-7.
15. Reits EA, Benham AM, Plougastel B, Neefjes J, Trowsdale J. Dynamics of proteasome distribution in living cells. EMBO J 1997; 16:6087-94.
16. von Mikecz A, Chen M, Rockel T, Scharf A. The nuclear ubiquitin-proteasome system: visualization of proteasomes, protein aggregates and proteolysis in the cell nucleus. Methods Mol Biol 2008; 463:191-202.
17. Lafarga M, Berciano MT, Pena E, Mayo I, Castano JG, Bohmann D, et al. Clastosome: a subtype of nuclear body enriched in 19S and 20S proteasomes, ubiquitin and protein substrates of proteasome. Mol Biol Cell 2002; 13:2771-82.
18. Carmo-Fonseca M, Berciano MT, Lafarga M. Orphan nuclear bodies. Cold Spring Harb Perspect Biol 2010; 2:000703.
19. Rockel TD, Stuhlmann D, von Mikecz A. Proteasomes degrade proteins in focal subdomains of the human cell nucleus. J Cell Sci 2005; 118:5231-42.
20. Heun P. SUMOrganization of the nucleus. Curr Opin Cell Biol 2007; 19:350-5.
21. Pines J, Lindon C. Proteolysis: anytime, any place, anywhere? Nat Cell Biol 2005; 7:731-5.
22. Tao LZ, Cheung AY, Nibau C, Wu HM. RAC GTPases in tobacco and Arabidopsis mediate auxin-induced formation of proteolytically active nuclear protein bodies that contain AUX/IAA proteins. Plant Cell 2005; 17:2369-83.
23. Floyd ZE, Trausch-Azar JS, Reinstein E, Ciechanover A, Schwartz AL. The nuclear ubiquitin-proteasome system degrades MyoD. J Biol Chem 2001; 276:22468-75.
24. Lenk U, Sommer T. Ubiquitin-mediated proteolysis of a short-lived regulatory protein depends on its cellular localization. J Biol Chem 2000; 275:39403-10.
25. Karni-Schmidt O, Zupnick A, Castillo M, Ahmed A, Matos T, Bouvet P, et al. p53 is localized to a sub-nucleolar compartment after proteasomal inhibition in an energy-dependent manner. J Cell Sci 2008; 121:4098-105.
26. Wade M, Wang YV, Wahl GM. The p53 orchestra: Mdm2 and Mdmx set the tone. Trends Cell Biol 2010; 20:299-309.
27. Gonzalez F, Delahodde A, Kodadek T, Johnston SA. Recruitment of a 19S proteasome subcomplex to an activated promoter. Science 2002; 296:548-50.
28. Koues OI, Dudley RK, Mehta NT, Greer SF. The 19S proteasome positively regulates histone methylation at cytokine inducible genes. Biochim Biophys Acta 2009; 1789:691-701.
29. Truax AD, Koues OI, Mentel MK, Greer SF. The 19S ATPase S6a (S6'/TBP1) regulates the transcription initiation of class II transactivator. J Mol Biol 2010; 395:254-69.
30. Mayr J, Wang HR, Nederlof P, Baumeister W. The import pathway of human and Thermoplasma 20S proteasomes into HeLa cell nuclei is different from that of classical NLS-bearing proteins. Biol Chem 1999; 380:1183-92.
31. Wang HR, Kania M, Baumeister W, Nederlof PM. Import of human and Thermoplasma 20S proteasomes into nuclei of HeLa cells requires functional NLS sequences. Eur J Cell Biol 1997; 73:105-13.
32. Fahrenkrog B, Koser J, Aebi U. The nuclear pore complex: a jack of all trades? Trends Biochem Sci 2004; 29:175-82.
33. Harel A, Forbes DJ. Importin beta: conducting a much larger cellular symphony. Mol Cell 2004; 16:319-30.
34. Hetzer MW, Wente SR. Border control at the nucleus: biogenesis and organization of the nuclear membrane and pore complexes. Dev Cell 2009; 17:606-16.
35. Onischenko E, Weis K. Nuclear pore complex-a coat specifically tailored for the nuclear envelope. Curr Opin Cell Biol 2011; 23:293-301.
36. Guttinger S, Laurell E, Kutay U. Orchestrating nuclear envelope disassembly and reassembly during mitosis. Nat Rev Mol Cell Biol 2009; 10:178-91.
37. Fernandez-Martinez J, Rout MP. Nuclear pore complex biogenesis. Curr Opin Cell Biol 2009; 21:603-12.
38. Savulescu AF, Shorer H, Kleifeld O, Cohen I, Gruber R, Glickman MH, et al. Nuclear import of an intact preassembled proteasome particle. Mol Biol Cell 2011; 22:880-91.
39. Chan RC, Forbes DI. In vitro study of nuclear assembly and nuclear import using Xenopus egg extracts. Methods Mol Biol 2006; 322:289-300.
40. Macaulay C, Forbes DJ. Assembly of the nuclear pore: biochemically distinct steps revealed with NEM, GTP gamma S and BAPTA. J Cell Biol 1996; 132:5-20.
41. Rotem A, Gruber R, Shorer H, Shaulov L, Klein E, Harel A. Importin beta regulates the seeding of chromatin with initiation sites for nuclear pore assembly. Mol Biol Cell 2009; 20:4031-42.
42. Fried H, Kutay U. Nucleocytoplasmic transport: taking an inventory. Cell Mol Life Sci 2003; 60:1659-88.
43. Gorlich D, Pante N, Kutay U, Aebi U, Bischoff FR. Identification of different roles for RanGDP and RanGTP in nuclear protein import. EMBO J 1996; 15:5584-94.

44. Effantin G, Rosenzweig R, Glickman MH, Steven AC. Electron microscopic evidence in support of alpha-solenoid models of proteasomal subunits Rpn1 and Rpn2. J Mol Biol 2009; 386:1204-11.
45. Kajava AV. What curves alpha-solenoids? Evidence for an alpha-helical toroid structure of Rpn1 and Rpn2 proteins of the 26 S proteasome. J Biol Chem 2002; 277:49791-8.
46. Henderson BR, Fagotto F. The ins and outs of APC and beta-catenin nuclear transport. EMBO Rep 2002; 3:834-9.
47. Jaggi RD, Franco-Obregon A, Muhlhausser P, Thomas F, Kutay U, Ensslin K. Modulation of nuclear pore topology by transport modifiers. Biophys J 2003; 84:665-70.
48. Kutay U, Izaurralde E, Bischoff FR, Mattaj IW, Gorlich D. Dominant-negative mutants of importin-beta block multiple pathways of import and export through the nuclear pore complex. EMBO J 1997; 16:1153-63.
49. Wu J, Matunis MJ, Kraemer D, Blobel G, Coutavas E. Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain, and a leucine-rich region. J Biol Chem 1995; 270:14209-13.
50. Pichler A, Gast A, Seeler JS, Dejean A, Melchior F. The nucleoporin RanBP2 has SUMO1 E3 ligase activity. Cell 2002; 108:109-20.
51. Forbes DJ, Kirschner MW, Newport JW. Spontaneous formation of nucleus-like structures around bacteriophage DNA microinjected into Xenopus eggs. Cell 1983; 34:13-23.
52. Lohka MJ, Masui Y. Formation in vitro of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. Science 1983; 220:719-21.
53. Cabrera R, Sha Z, Vadakkan TJ, Otero J, Kriegenburg F, Hartmann-Petersen R, et al. Proteasome nuclear import mediated by Arc3 can influence efficient DNA damage repair and mitosis in *Schizosaccharomyces pombe*. Mol Biol Cell 2010; 21:3125-36.
54. Funakoshi M, Tomko RJ Jr, Kobayashi H, Hochstrasser M. Multiple assembly chaperones govern biogenesis of the proteasome regulatory particle base. Cell 2009; 137:887-99.
55. Hendil KB, Kriegenburg F, Tanaka K, Murata S, Lauridsen AM, Johnsen AH, et al. The 20S proteasome as an assembly platform for the 19S regulatory complex. J Mol Biol 2009; 394:320-8.
56. Kaneko T, Hamazaki J, Iemura S, Sasaki K, Furuyama K, Natsume T, et al. Assembly pathway of the Mammalian proteasome base subcomplex is mediated by multiple specific chaperones. Cell 2009; 137:914-25.
57. Roelofs J, Park S, Haas W, Tian G, McAllister FE, Huo Y, et al. Chaperone-mediated pathway of proteasome regulatory particle assembly. Nature 2009; 459:861-5.