Protein Synthesis upon Acute Nutrient Restriction Relies on Proteasome Function

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The mechanisms that protect mammalian cells against amino acid deprivation are only partially understood. We found that during an acute decrease in external amino acid supply, before up-regulation of the autophagosomal-lysosomal pathway, efficient translation was ensured by proteasomal protein degradation. Amino acids for the synthesis of new proteins were supplied by the degradation of preexisting proteins, whereas nascent and newly formed polypeptides remained largely protected from proteolysis. Proteasome inhibition during nutrient deprivation caused rapid amino acid depletion and marked impairment of translation. Thus, the proteasome plays a crucial role in cell survival after acute disruption of amino acid supply.

References and Notes
7. Materials and methods are available as supporting information on Science Online.

Supporting Online Material
www.sciencemag.org/cgi/content/full/310/5756/1957/DC1

SOM Text
Figs. S1 to S4
Tables S1 and S2
References
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Fig. 1. Proteasome activity is required to sustain protein synthesis upon amino acid restriction. (A) Amounts of Ub-EGFP reporter protein were analyzed in transiently transfected HeLa cells at 0, 5, and 10 min after addition of dimethyl sulfoxide (DMSO) alone, 5 mM CHX, 100 μM MG132 in DMSO, or CHX and MG132 combined by anti-GFP immunoblotting. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. (B) Translation was followed by measuring the incorporation of 35S-Met into TCA-insoluble material (left) or analyzing newly synthesized proteins by 10% SDS-PAGE and phosphorimaging (right). At time 0, 50 μCi/ml 35S-Met and MG132 were added. Circles, normal medium (80 μM Leu); squares, deficient medium (0.8 μM Leu). Solid symbols, MG132 addition; open symbols, DMSO controls. (C) Translation was analyzed as in (B). In addition to MG132, 40 μM clasto-lactacystin-β-lactone (CL-β-lactone) or 40 μM epoxomicin were used, and translation was observed for 15 min (fig. S1). (A and B). Protein synthesis is expressed in percentage of controls lacking inhibitor. Means ± SD of three independent experiments are shown. Gray bars, normal medium; black bars, Leu-deficient medium. (D) Same as (B), except that 10 mM Phe was varied from 20 μM (normal medium) to 0.2 μM (deficient medium) and labeling was with 100 μCi/ml 3H-Leu. Representative results of at least three independent experiments are shown.

The proteasome was simultaneously inhibited (Fig. 3A). This is indicative of a physiologically relevant depletion of the intracellular leucine pool under these conditions. Efficient translation resumed rapidly upon re-addition of the lacking amino acid, even when the block of proteasome function was maintained (Fig. 3B). Amino acid analysis demonstrated directly that the proteasome supplied building blocks for protein synthesis. The addition of translation inhibitor (CHX) to cells growing in normal medium resulted in a measurable increase in intracellular leucine within 10 min (table S1). This effect was less pronounced in the presence of the proteasome inhibitor. In leucine-deficient medium, intracellular leucine was only detectable upon addition of CHX when the proteasome was not inhibited (table S1), indicating that combined amino acid deficiency and proteasome inhibition severely depleted the intracellular amino acid pool.

At least 30% of newly synthesized proteins are thought to be degraded by the proteasome during and immediately after translation, presumably reflecting a general inefficiency of protein biosynthesis and folding (7). How...
Fig. 3. Immediate cellular effects of amino acid restriction and proteasome inhibition. (A) Activation of GCN2 kinase in HeLa cells by reducing the medium concentration of Leu from 80 to 0.8 μM and simultaneous proteasome inhibition. Activated GCN2 kinase (pGCN2) was detected by immunoblotting with an antibody to pGCN2. A asterisk, nonspecific band. Equal loading was confirmed with antibodies detecting GCN2 independent of its phosphorylation (GCN2). At time 0, 100 μM MG132 or DMSO was added. (B) Translation was analyzed in Leu-deficient medium (–Leu) or Phe-deficient medium (–Phe). Incorporation of 35S-Met into TCA-precipitable material was measured. 50 μCi/ml 35S-Met was added either together with MG132 (solid symbols) or with DMSO (open symbols). After 10 min (dashed line), the respective lacking amino acid (triangles) or control amino acid (circles) (Phe in case of –Leu medium, Leu in case of –Phe medium) was added to normal concentration. aa, amino acid. Representative results of at least three independent experiments are shown.

Fig. 4. Effect of proteasome activity on radiolabeling of newly synthesized proteins and degradation of faulty proteins. (A and B) Newly synthesized proteins in HeLa cells were labeled with 50 μCi/ml 35S-Met in Met-deficient medium (A), or with 100 μCi/ml 3H-Leu in Leu-deficient medium (B). Radioactivity of TCA-insoluble material was measured. MG132 (solid symbols) or DMSO (open symbols) were added with radioactive tracers at time 0. (Right) Protein synthesis after 8 min (A) or 10 min (B) of labeling is expressed in percentage of controls lacking inhibitor. Means + SD of three independent experiments are shown. (C) Labeling with 50 μCi/ml 35S-Met in normal medium lacking nonessential amino acids. Circles, cells in normal medium; squares, cells preincubated for 15 min in 20 mM L-azetidine-2-carboxylic acid (Azc) before and during labeling. Open symbols, DMSO; solid symbols, MG132-containing cultures. cpm, counts per minute. (D) Proteins were labeled as in (C). Gray bars, control cells; black bars, Azc-treated cells. After labeling for 10 min, a chase was performed with 20 mM unlabeled Met. Half of the cultures received 100 μM MG132 and 10 μM clasto-lactacystin-β-lactone during labeling and chase (+ proteasome inhibitor), and the other half received DMSO (– proteasome inhibitor). TCA-precipitable radioactivity at time 0 was set as 100%. Means + SD of three experiments are shown.

For labeling, cells are usually preincubated in media lacking the respective nonradioactive amino acid to increase the incorporation of radiolabel into newly made proteins (7). Based on our findings, preincubation with a proteasome inhibitor (7) should enhance this effect as a result of severe intracellular amino acid depletion (table S1 and Fig. 3A). Indeed, the incorporation of 35S-Met into newly synthesized protein increased more than 10-fold when labeling was performed in methionine-deficient medium (Fig. 4A). As predicted, proteasome inhibition during amino acid starvation resulted in a substantial further increase in incorporated radioactivity (Fig. 4A), even though the efficiency of translation was reduced (Fig. 1). This effect was independent of the specific ratio of labeled-to-unlabeled amino acid (fig. S3) and was equally observed when cells were labeled with 3H-Leu in leucine-deficient medium (Fig. 4B). Double-labeling experiments with 3H-Leu and 35S-Met in methionine-deficient medium showed that proteasome inhibition increased the incorporation of 35S-Met into equal amounts of newly synthesized, 3H-Leu-labeled protein by twofold (fig. S4A). A threefold increase in 35S-Met mRNA was detected in cells after 15 min of proteasome inhibition, which would explain the increased incorporation of 35S-Met into newly formed protein (fig. S4B). Thus, the higher incorporation of radiolabel observed upon proteasome inhibition in amino acid deficient medium was due to an increase in specific radioactivity of the intracellular amino acid pool, not to the stabilization of a large fraction of newly synthesized proteins. At most, only a few percent of total protein was rapidly degraded immediately upon translation in the cell types analyzed here.

To test whether translating polypeptides remain protected against proteasomal degradation even when unable to fold, we incubated cells in the presence of the proline analog L-azetidine-2-carboxylic acid (Azc). Whereas the addition of Azc resulted in a reduced incorporation of 35S-Met, no notable additional accumulation of radiolabeled protein was detectable upon proteasome inhibition within 12 min (Fig. 4C). This suggested that removal by the proteasome of misfolded proteins containing Azc occurred only after a substantial lag period. To address this possibility, cells were labeled with 35S-Met for 10 min, followed by a chase with excess unlabeled methionine. More than 40% of the proteins synthesized in the presence of Azc were degraded within 60 min and this effect was largely prevented by proteasome inhibition (Fig. 4D). The extent of protein degradation observed in normal medium (~20% over 60 min) is in agreement with previous studies demonstrating the proteasomal turnover of short-lived proteins (23, 24). Thus, when cells produce a substantial amount of protein chains...
Human memory can be characterized as an elaborate network of stored representations (1, 2). Recalling a particular event involves reactivating the constellation of representations that was active during that event, a phenomenon that Tulving has referred to as “mental time travel” (3). One of the major puzzles of human memory is how we enact this process of mental time travel. More concretely: When we are instructed to recall a particular event, how do we manage to select representations corresponding to that event, as opposed to representations from other events (4, 5)?

Several theorists have argued that recalling an event involves a process of contextual reinstatement (6, 7). When asked to recall memories of a certain type, a person activates knowledge about the general properties of those events and then uses this general knowledge to constrain the search for memories of the target events. For example, in trying to remember a trip to the zoo, a person could use their general knowledge of the kinds of animals that are typically found at zoos as a contextual cue for specific memories of seeing those animals. If specific details are recalled, these details can be used to further refine the retrieval cue, which leads to recall of additional details, and so on. Over time, the person continues to probe memory, and the set of representations that are active at recall increasingly comes to resemble the set of representations that were active during the targeted event. Whereas a number of behavioral memory studies have found evidence consistent with the contextual reinstatement hypothesis (8–10), this kind of evidence is necessarily indirect. We can infer (based on theoretical grounds) that the observed patterns of behavioral data arise from increased match between cues at test and stored memory traces, but these studies do not directly measure cue-trace match.

We used functional magnetic resonance imaging (fMRI) to more directly test the contextual reinstatement hypothesis. In neural terms, the contextual reinstatement hypothesis leads to a number of predictions. The most basic prediction is that, when subjects try to recall specific details from a particular episode or type of episode, the pattern of brain activity (during recall) will progressively come to resemble the pattern of activity that was present during the to-be-remembered episode. Furthermore, it should be possible to relate the reinstatement of
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