A Mechanism Regulating Proteolysis of Specific Proteins during Renal Tubular Cell Growth

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Abstract

Growth factors suppress the degradation of cellular proteins in lysosomes in renal epithelial cells. Whether this process also involves specific classes of proteins that influence growth processes is unknown. We investigated chaperone-mediated autophagy, a lysosomal import pathway that is dependent on the 73 kDa heat shock cognate protein (hsc73) and allows the degradation of proteins containing a specific lysosomal import consensus sequence (KFERQ motif). Epidermal growth factor (EGF) or ammonia, but not transforming growth factor β1 (TGF-β), suppresses total protein breakdown in cultured NRK-52E renal epithelial cells. EGF or ammonia prolonged the half-life of glyceraldehyde-3-phosphate, a classic substrate for chaperone mediated autophagy, by more than 90%, while TGF-β1 did not. EGF caused a similar increase in the half-life of the KFERQ-containing, paired box-related transcription factor, Pax2. The increase in half-life was accompanied by an increased accumulation of proteins with a KFERQ motif, including glyceraldehyde-3-phosphate and Pax2. Ammonia also increased the level of Pax2 protein. Lysosomal import of KFERQ proteins depends on the abundance of the 96 kDa lysosomal glycoprotein protein (lgp96), and we found that EGF caused a significant decrease in lgp96 in cellular homogenates and associated with lysosomes. We conclude that EGF in cultured renal cells regulates the breakdown of proteins targeted for destruction by chaperone mediated autophagy. Since suppression of this pathway results in an increase in Pax2, these results suggest a novel mechanism for regulation of cell growth.

Keywords:
EGF, ammonia, Lysosome, Autophagy, Pax2, glyceraldehyde-3-phosphate.
**Introduction**

A major response of cells to growth factors is a generalized increase in protein synthesis, including the synthesis of specific classes of proteins (1). In addition to controlling synthesis, growth factors can suppress the bulk degradation of proteins (2). For example, in renal tubular epithelial cells, we found that EGF suppresses the breakdown of the mass of intracellular proteins (3). Suppression of proteolysis in response to growth factors involved decreased lysosomal degradation rather than decreased proteasomal or calcium-sensitive proteases (3). Despite reports that proteolysis is regulated, no one has determined if specific classes of proteins are being regulated by growth factors.

Lysosomes degrade extracellular proteins (via endocytosis), membrane proteins and organelles (via autophagy), and can degrade cytosolic proteins via direct import through the lysosomal membrane (4, 5). Dice and colleagues showed that there is a specific import pathway involving the 73 kDa heat shock cognate protein (hsc-73)\(^1\), called chaperone-mediated autophagy (6). Hsc73 binds to a penta-peptide motif (consensus sequence: KFERQ) on the target protein and, acting as a chaperone, unfolds the target protein (7). Hsc73 bound to the substrate protein then interacts with an intrinsic lysosomal membrane protein, the 96 kDa lysosomal glycoprotein (lgp96, also called lysosomal membrane protein 2a (Lamp2a)) (8). After recruiting other accessory proteins, the target protein is transported through the lysosomal membrane and degraded (9). Dice and colleagues also showed that chaperone mediated autophagy can be regulated by calorie deprivation which accelerates the proteolysis of proteins with KFERQ motifs in lysosomes from liver (10). In kidney and liver, up to 30% of proteins contain the KFERQ motif, including many of the proteins involved in glycolysis. Since most glycolytic proteins have long half-lives, an increase in degradation could function to down regulate their abundance.
Since we found that growth factors suppress lysosomal proteolysis in renal cells, we wanted to determine if growth factors regulate the half-life of proteins which are substrates for chaperone-mediated autophagy. In pursuing this question, we uncovered a novel mechanism that leads to accumulation of specific proteins involved in the regulation of cellular growth.

**Experimental procedures**

All chemicals or reagents were purchased from Sigma Chemical (St. Louis, MO), except Dulbecco's Modified Eagle Media (DMEM), newborn calf serum, Trypsin - EDTA, and Penicillin - Streptomycin which were obtained from GIBCO (Grand Island, NY). Recombinant human TGF-β1 and EGF were obtained from R&D Systems (Minneapolis, MN), and L-[U-14C] phenylalanine from New England Nuclear, Dupont (Boston, MA). Anti-hsc73 antisera was purchased from Maine Biotechnology (Portland, ME), anti-M2 pyruvate kinase from Scebo-Tech, A.G. (Wettenburg, Germany), anti-GAPDH from Bodesign Int. (Kennebunk, ME). Anti-Pax2 sera from Zymed (S. San Francisco, CA). Affinity purified anti-sera to the penta-peptide KFERQ and to lgp96 were a generous gift of J. F. Dice (Tufts University). Anti-hexokinase sera was a gift of E. Knecht (Universidad de Vallencia, Spain).

NRK-52E cells (a rat kidney epithelial cell line(11)) were obtained from ATCC (Bethesda, MD) at passage 15, subcultured, and grown in high glucose DMEM supplemented with 25 mM HEPES, 25 mM glutamine, and 5% calf serum. Studies were performed on cells from passages 19-29. Cells in 6-well plates were grown to confluence and rendered quiescent by serum removal 48
hours prior to experimental treatment. The cell culture media was refreshed every 24 hours to maintain a constant pH; it did not differ between control and treatment groups.

Recombinant human TGF-β1 was reconstituted in 4 mM HCl containing 0.1% heat-treated bovine serum albumin. Recombinant human EGF was reconstituted in PBS containing 0.1% heat-treated bovine serum albumin. In all studies, TGF-β1 was used at 10^{-10} M, EGF at 10^{-8} M and NH₄Cl at 10 mM concentrations (12); the appropriate vehicle was added to control cells.

Measurements of growth and protein turnover.

After exposure to an experimental variable, cells were washed with PBS, incubated with 0.05% trypsin/0.5 mM EDTA for 5 minutes, centrifuged at 1500 x g for 5 minutes, and washed with PBS. The final pellet was resuspended in 1 ml of 50 mM Na₂PO₄ (pH 7.4) and lysed on ice by repeated passage though a 27 gauge needle. The lysate was divided and stored at -70° C for protein and DNA determination as described (12).

Protein degradation was measured as the release of L-[U-¹⁴C] phenylalanine from cells pre-labeled as described (3-13). Briefly, 5 mM of unlabeled phenylalanine was added to the media to minimize reuse of phenylalanine released by protein breakdown and an initial 4 hour washout period was used to eliminate short-lived proteins and unincorporated L-[¹⁴C] phenylalanine. Aliquots of media were removed at intervals, treated with TCA to remove protein, and radioactivity determined. At the end of the experiment, cell protein was solubilized in 1 ml/well of 1% sodium dodecyl sulfate (SDS) and the remaining radioactivity was measured. The protein degradation rate was calculated as the slope of the logarithm of [¹⁴C] phenylalanine remaining in cell protein versus time.
Turnover of Specific Proteins

Confluent cells in 100 mm dishes were incubated with 100 μCi L-[35S] cystine/methionine (ICN, Costa Mesa, CA). For glyceraldehyde 3 phosphate dehydrogenase (GAPDH), the labeling was performed in serum-free DMEM with cold cystine/methionine present for 72 hours. For Pax2, cells were treated with EGF in cystine/methionine free media for 20 hours to increase the labeling of Pax2 because its abundance is very low in quiescent cells. After 2 washes in serum free media, a four hour washout in serum and growth factor free, cystine/methionine-containing media was performed prior to addition of growth factors. Subsequently, cells were washed twice with serum free media before adding the experimental variable in media containing an excess of cold cystine and methionine. Culture media were always changed daily with an additional wash. At time zero and at various times up to 72 hours for GAPDH and up to 24 hours for Pax2, cells were lysed in a 1% NP40 lysis buffer containing 100 mg/ml PMSF, 2 mM sodium EDTA, 4 mg/ml aprotinin, 2 mg/ml leupeptin, and 2 mg/ml pepstatin. One mg/ml anti- GAPDH or Pax2 anti-sera was added to equal amounts of cellular protein which was precipitated with try protein G sepherose beads. After 3 washes with lysis buffer, the immunoprecipitate was separated by SDS PAGE, underwent autoradiography, and was quantitated by use of the Signmagel program. Protein half-life was calculated from the slope of the logarithmic transformation of the densitometry data plotted against time. We documented completeness of recovery by performing western blots on the supernatants after immunoprecipitation (data not shown).

Western Blotting
Cells in 60 mM tissue culture dishes were washed twice in ice cold PBS and lysed in a buffer containing 100 g/ml PMSF, 2 mM sodium EDTA, 2 g/ml aprotinin, 2 g/ml leupeptin, and 2 g/ml pepstatin. After centrifugation, protein in the supernatant was determined and the supernatant was boiled in buffer containing 1% SDS, 0.5% -mercaptoethanol, proteins separated by SDS-PAGE, transferred to nitrocellulose filters, and 5% fat free milk protein or 3% BSA was used as blocking reagents. Antibodies were detected using the ECL system (Amersham, Arlington Heights, IL) and Kodak BCL film.

Lysosome Isolation

Lysosomes were isolated as described by Cuervo et al. (14). Briefly, cells in two 25 cm² plates per group were washed in ice cold PBS and then homogenized after scraping in ice cold buffer (2.5 mM tris (pH 7.2), 0.25 M sucrose) by 20 strokes of a Teflon Polytron homogenizer at 4°C. One gram protein per 7 ml 0.25 M sucrose was centrifuged at 2500 g for 10 minutes, the post nuclear supernatant was placed on a discontinuous gradient of 35% and 17% metrizamide (pH 7.0) and 6% percoll and centrifuged at 6800 g for 25 minutes. The lysosome/mitochondrial fraction at the metrizamide/percoll interface was resuspended to a final concentration of 57% metrizamide. On top of this fraction, there was a discontinuous metrizamide gradient of equal volumes of 35%, 17%, and 5%, metrizamide with a final 0.25% sucrose layer. This gradient was centrifuged for 1 hours at 95,000 g. The lysosomes sediment to the interface of 5%-17% metrizamide and mitochondria at the 35-57% interface. Purity of the lysosomal or mitochondrial fractions was determined by the activity of -N-hexosaminidase (lysosome) and mitochondrial succinic dehydrogenase and by the presence of lgp96 (15).
Results are expressed as mean ± SEM. Because there was experiment-to-experiment variation in the magnitude of responses, results are presented as percentage of the control value determined simultaneously. Differences between two groups were analyzed by Student’s T test, but multiple comparisons were analyzed by analysis of variance. Comparisons of slopes of lines representing the release of L-[U-14C] phenylalanine were done by analysis of co-variance.

Results

We treated NRK-52E cells with growth factors and found different growth properties (figure 1): EGF causes hyperplasia (increased DNA content), and increases (~30%) the half-life of long lived proteins. TGF β increases in the protein to DNA ratio (hypertrophy), but does not significantly suppress proteolysis. The combination of EGF plus TGF β causes hypertrophy with suppression of proteolysis, while ammonia causes less hypertrophy, even though there is even greater suppression of proteolysis. We have previously shown that EGF, TGF β and EGF plus TGF β increase protein synthesis, while ammonia does not affect synthesis (12, 13, 16).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has a KFERQ consensus sequence (17) and is a classic substrate for chaperone mediated autophagy (15, 18). As shown in figure 2 and table 1, the half-life of GAPDH measured in a pulse-chase experiment increased by ~ 90% in cells treated with either EGF or ammonia, but did not significantly change in cells treated with TGF β alone.
We also tested whether these agents change the abundance of specific proteins with the KFERQ lysosomal import sequence. GAPDH abundance increased with treatments that suppress proteolysis, but TGF-β 1, which does not affect proteolysis, did not increase GAPDH. We also examined the M₂ isoform of pyruvate kinase because it is a glycolytic enzyme with a KFERQ consensus sequences (19), and it binds hsc73 and is imported into lysosomes (6). In contrast, hexokinase is a glycolytic enzyme that lacks a KFERQ sequence (15). Conditions that stimulate cell growth also increase the abundance of M₂ isoform of pyruvate kinase as well as other proteins recognized by anti-KFERQ affinity-purified sera (figure 3). Hexokinase abundance did not increase with any of the growth factors. Although the pattern of changes was similar between proteins recognized by anti-KFERQ sera and M₂ isoform of pyruvate kinase, the magnitude of increase in M₂ isoform of pyruvate kinase was much greater, suggesting that its synthesis is also stimulated (figure 3b, 3c).

Glycolytic enzymes are not the only proteins containing KFERQ sequences which may be important intermediates influencing renal cell growth. For example, the renal, paired-box related transcription factor, Pax2, contains a conserved KFERQ sequence at amino acids 38 to 42 (20). We found that the half-life of Pax2 (figure 4) also increased with EGF treatment, and that the abundance of Pax2 was increased by growth factors; the smallest rise occurred with TGF-β 1. Interestingly, Pax2 abundance also increased when lysosomal proteolysis was inhibited with NH₄Cl (figure 3d). Thus different stimuli causing growth also increase Pax2 abundance.

To determine the mechanisms that control suppression of proteolysis, we examined if the regulatory proteins of chaperone mediated autophagy change in response to growth factors. Hsc73 did not change in abundance (figure 3a). We also examined the lysosomal membrane receptor for
protein translocation, lgp96, in lysates and in association with isolated lysosomes, using sera
directed against the 12 amino acid cytoplasmic portion of lgp96 that binds to hsc73 (8). The quality
of lysosomes isolated did not vary between control and EGF treated cells as assayed by
hexosaminidase activity (Table 2). Isolated lysosomes exhibited immunostaining for lgp96 (figure
5a): the level was seven- fold higher than in whole cell lysates (Figures 5b). Lgp96 was not detected
in the mitochondrial fractions. In whole cell lysates, lgp96 abundance decreased by 30-40% after
24 or 96 hours of treatment with stimuli that suppress proteolysis (figure 5c, e). In contrast, TGF-
1, which did not affect proteolysis, did not affect lgp96 levels. Since lysosomal associated lgp96,
correlates more closely with activity of chaperone-mediated autophagy than total cellular lgp96 (21),
we examined lysosomal associated lgp96 with EGF treatment and found a 48±10% (p<0.05, n=3)
decrease compared to lysosomes isolated from control cells (Figure 5d, e).

Discussion

In the early 1980's, it was recognized that specific growth factors and activated oncogenes
could suppress protein degradation in certain cell types, including epithelial cells (22). It was not
known, however, which classes of proteins develop longer half-lives during growth, nor how this
response was regulated. We found that EGF suppresses the breakdown of the bulk of proteins in
NRK-52E cells by a mechanism that involved suppression of lysosomal function, but not proteolysis
by proteasomal or calcium-activated proteases (3).

Physiologic conditions can regulate specific pathways of lysosomal proteolysis. For
example, calorie deprivation increases the degradation of proteins with a KFERQ motif in liver and
kidney lysosomes (10). How does this finding bear on growth factor-induced renal cell growth? Conditions stimulating renal cell growth increase glycolysis, and many glycolytic enzymes contain KFERQ motifs (17, 23-25). Thus, by acting in the opposite fashion as calorie deprivation, growth factors could suppress the degradation of glycolytic enzymes and contribute to the increase in glycolysis that accompanies renal growth. Our results confirm that EGF acts to prolong the half-life of the classic substrate for chaperone-mediated autophagy, GAPDH, and increase the abundance of KFERQ-containing proteins.

Our results provide additional insights into the relationship among growth factors, cell growth and lysosomal protein degradation. Firstly, only specific growth factors influence lysosomal function. For example, EGF clearly stimulates cell growth and suppresses total proteolysis and the proteolysis of substrates of chaperone-mediated autophagy. In contrast, TGFβ caused the smallest increase in growth and has almost no effect on proteolysis. We do not conclude that suppression of proteolysis is the sole mechanism causing KFERQ-containing protein accumulation, since the accumulation of KFERQ proteins that occurred with TGFβ treatment almost certainly reflects increased synthesis (figures 3b and 3c).

Secondly, our results show that regulation of this lysosomal pathway by growth factors leads to prolongation of the half-life of Pax2, which has been implicated in renal cell growth in development, cyst formation, and renal cell carcinoma (20, 26). Because there is also an increase in the abundance of Pax2 in cells treated with EGF and because EGF causes only trivial increases in Pax2 mRNA in renal tubular cells (27), the increase in half-life we found could be physiologically relevant. Since Pax2 acts as a transcription factor, these responses suggest a new mechanism by which growth factors regulate cell growth: not only do they suppress the degradation
of the bulk of cytoplasmic proteins (3), but they increase the availability of at least one critical transcription factor.

Finally, our results provide unexpected information about a potential mechanism by which ammonia could increase cell growth. The growth of renal cells characteristically found in response to metabolic acidosis is attributed to ammonia which can reach concentrations as high as 5mM in the cortex of the kidney (28). Ammonia had been thought to act only by changing lysosomal pH and nonspecifically suppressing lysosomal proteolysis leading to accumulation of cytosolic proteins (16, 29). However, our results suggest that ammonia also acts by suppressing degradation of specific signaling proteins such as Pax2. The upregulation of transcription factors could allow expression of particular proteins important for growth without an increase in global protein synthesis.

Regarding the mechanism involved in changing lysosomal degradation, we and others find that the abundance of hsc73 does not change even when activity of this pathway changes (9;14). Curiously, hsc73 contains KFERQ sequences but is resistant to degradation within hepatic lysosomes responding to starvation (9). On the other hand, we did observe a decrease in the abundance of lgp96 including a sharp decrease in the amount of lgp96 specifically associated with lysosomes (figure 5). This finding is consistent with the close correlation between lgp96 associated with lysosomes and the activity of chaperone-mediated autophagy (30), (14, 21).

Although there are similarities between the effects of EGF and ammonia on proteolysis of KFERQ containing proteins and lgp96 levels, there are differences in their actions on lysosomes. We found that pharmacologic agents that specifically inhibits lysosomal proteolysis (ammonia, methylamine, bafilomycin A1 or leupeptin plus the protease inhibitor, E64) convert the cellular proliferation in response to EGF into hypertrophy (31). The change in lgp96 abundance may be a
common pathway increasing growth promoting proteins such as glycolytic enzymes and Pax2, while an additional influence of lysosomal inhibitors may account for the conversion of hyperplasia to hypertrophy.

Besides regulation of chaperone-mediated autophagy, EGF could affect the function of other pathways of lysosomal proteolysis. Autophagy may also be regulated by growth factors (32) leading to slower degradation of organelles and membranes. EGF acts through phosphoinositide-3 kinase as it suppresses proteolysis in renal cells (Franch and Du, unpublished observation), and phosphoinositide kinase has been reported to regulate autophagy in cultured liver cells (33).

One practical prediction of these results is that a KFERQ sequence may be used to identify proteins that are up-regulated during renal cell growth. Besides glycolytic enzymes, there are a large number of proteins in the NCBI Entrez database which contain conserved KFERQ sequences and that are important for renal tubule cell growth. These include enzymes involved in phospholipid metabolism (choline kinase (Genebank accession # 139962) and phosphorylcholine transferase (34)), ion transporters (subunit of the Na/K ATPase (35)), and signaling molecules such as Pax2 (20). The KFERQ sequence is present in the Pax isoforms expressed in the urinary tract (Pax 2, 5 and 8), but not in other Pax isoforms, suggesting that the link between Pax proteins and this proteolytic pathway may be specific to the urinary tract (36, 37). Finally, the signaling proteins MARKS and IκB have been shown to have their abundance regulated by this pathway (30, 38).

**Footnotes**

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Abbreviations: EGF, Epidermal growth factor; TGF-β, transforming growth factor-β; hsc73, 73 kDa heat shock cognate protein; lgp96, 96 kDa lysosomal glycoprotein; DMEM, Dulbecco's Modified Eagle Media; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase;
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Table 1. Growth factors change GAPDH protein half-life. NRK-52E cells were radiolabeled when quiescent, growth factors were added during a cold chase period, and then GAPDH was immunoprecipitated at different times. Protein half-life was calculated from the slope of the logarithmic transformation of the densitometry of autoradiograms plotted against time. \(^*\) p<0.05 vs. Control, n=4-7.

|                | Half-life (hrs) | Percentage of Control Half-life |
|----------------|----------------|--------------------------------|
| Control        | 38.1±5.3       | 100%                           |
| EGF (10\(^{-8}\) M) | 74.9±3.1\(^*\) | 196%                           |
| TGF (10\(^{-10}\) M) | 48.6±3.8      | 128%                           |
| Ammonia 10 mM  | 73.7±10.8\(^*\) | 193%                           |
| Control (N=3)        | Hexosaminidase Activity % | Protein % | Specific Activity |
|----------------------|---------------------------|-----------|-------------------|
| Homogenate           | 100                       | 100       | 1                 |
| Post-Nuclear         | 28.0±8.6                  | 14.4±1.1  | 1.95±0.61         |
| Supernatant          |                           |           |                   |
| Mitochondria         | 0.40±0.15                 | 0.49±0.26 | 0.92±0.19         |
| Lysosomes            | 2.55±0.38                 | 0.80±0.01 | 3.19±0.48         |
| EGF (N=3)            |                           |           |                   |
| Homogenate           | 100                       | 100       | 1                 |
| Post-Nuclear         | 16.4±4.0                  | 12.3±0.7  | 1.36±0.41         |
| Supernatant          |                           |           |                   |
| Mitochondria         | 0.87±0.42                 | 0.54±0.16 | 1.52±0.34         |
| Lysosomes            | 1.60±0.25                 | 0.58±0.18 | 2.93±0.50         |

Table 2. Hexosaminidase activity in isolated lysosomes. NRK-52E cells were grown as in figure 1 and lysosomes and mitochondria isolated by metrizamide density gradient centrifugation. There are no significant differences between control and EGF. *p<0.05 vs. control.
Figure legends:

1. Growth factors exert different effects on growth and proteolysis. NRK-52E cells were treated with EGF \( (10^{-8}\text{M}) \), TGF 1 \( (10^{-10}\text{M}) \), EGF+TGF 1, and NH\(_4\)Cl (10mM). A) Protein, DNA and protein to DNA levels after 72 hours of treatment are expressed as a percentage of the increase over the value measured in cells treated with the vehicle only. \( n=14-18 \). * \( p<0.05 \) vs. control, + \( p<0.05 \) vs. EGF alone and NH\(_4\)Cl alone. B) Protein degradation expressed as the slope of the log of the percentage of the counts remaining in cells at different times. The figure is a representative experiment of 3 repeats, \( n=6 \) per group. The slopes of all lines are significantly different from each other \( (p<0.05) \) except for control compared to TGF 1 and EGF compared to EGF+TGF 1.

2. Growth factors change glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein half-life. A. Autoradiogram of NRK-52E cell lysates after immuno-precipitation with anti-GAPDH in a pulse chase experiments. Cells were treated as in figure 1 except they were radiolabeled when quiescent. Lanes are labeled according to treatment (control, EGF or TGF 1) and duration of treatment (hours) after removal of L-\[^{35}\text{S}\] cystine/methionine. 0 represents initial labeling. Autoradiogram shown is representative of 5 repeats. B. Density of radioactivity

3. Protein abundance of extracts of NRK-52E cells treated for 96 hours as denoted in figure 1.
Panel A. Western analysis of 40 mg protein per lane with M2 pyruvate kinase, GAPDH, hexokinase and anti-KFERQ peptide antibodies or sera. A representative blots of 4 or more repeats. B. Quantification by densitometry of western blots for M2 pyruvate kinase. * p<0.05 vs. control, n=5. C. Quantification by densitometry of western blots for total KFERQ proteins * p<0.05 vs. control, n=9. D. Western analysis of 50 mg per lane with anti-Pax2 antibodies (Representative blot of 4 repeats).

4. EGF changes Pax2 protein half-life. Autoradiogram of NRK-52E cell lysates after immunoprecipitation with anti-Pax2 in a pulse chase experiment. Labeling with L-[\textsuperscript{35}S] cystine/methionine was performed with 10-8 M EGF present for 20 hours in cold cystine/methionine free media followed by a 4 hour washout in serum and EGF free media with cystine/methionine present before addition of EGF. Otherwise cells were grown as in figure 1. Lanes are labeled as the type and hours of treatment with EGF. 0 represents initial labeling. Autoradiogram shown is representative of 3 repeats.

5. Lgp 96 abundance by western analysis of protein abundance of RIPA extracts of NRK-52E cells and isolated lysosomes and mitochondria. Representative blots of 3-4 repeats. A. Lgp96 abundance in 10 ug of protein from isolated mitochondria (Mito) and lysosomes (Lyso). B. Lgp96 abundance in 50 g of protein from whole cell homogenates (Homo) and 7 mg lysosomal protein. C. Lgp96 abundance in 50 g of protein from cells treated for 96 hours as in figure 1. D. Lgp96 abundance in 5 mg of protein from isolated lysosomes in cells treated for 24 hours as
in figure 1. E. Densitometry of all repeats of the experiment shown in panel C (n=4) and D (n=4) expressed as a percentage of control values (dotted line). * p<0.05 vs. Control.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
A mechanism regulating proteolysis of specific proteins during renal tubular cell growth
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