The Notch Intracellular Domain Is Ubiquitinated and Negatively Regulated by the Mammalian Sel-10 Homolog*

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The Caenorhabditis elegans sel-10 protein is structurally similar to E3 ubiquitin ligases and is a negative regulator of Notch (lin-12) and presenilin signaling. In this report, we characterize the mammalian Sel-10 homolog (mSel-10) and analyze its effects on Notch signaling. We find that mSel-10 localizes to the cell nucleus, and that it physically interacts with the Notch 1 intracellular domain (IC) and reduces Notch 1 IC-mediated activation of the HES 1 promoter. Notch 1 IC is ubiquitinated by mSel-10, and ubiquitination requires the presence of the most carboxy-terminal region of the Notch IC, including the PEST domain. In the presence of the proteasome inhibitor MG132, the amount of Notch 1 IC and its level of ubiquitination are increased. Interestingly, this accumulation of Notch 1 IC in the presence of MG132 is accompanied by decreased activation of the HES 1 promoter, suggesting that ubiquitinated Notch 1 IC is a less potent transactivator. Finally, we show that mSel-10 itself is ubiquitinated and degraded by the proteasome. In conclusion, these data reveal the importance of ubiquitination and proteasome-mediated degradation for the activity and turnover of Notch ICs, and demonstrate that mSel-10 plays a key role in this process.

The Notch signaling pathway is important for cellular differentiation in many organs and tissues in most, if not all, multicellular species. A Notch receptor is a single transmembrane protein with a large extracellular domain containing multiple epidermal growth factor repeats, and an intracellular domain containing ankyrin repeats and PEST sequence. Notch receptors interact with cell-bound ligands of the DSL (Delta/Serrate/Lag-1) type presented on juxtaposed cells (1). Notch signaling requires at least three proteolytic cleavages of the receptor (for review, see Ref. 2). The two first cleavages take place at the extracellular side, whereas the third cleavage is controlled by presenilins and occurs in the transmembrane region close to the cytoplasmic side (3–6). The third cleavage releases the intracellular domain (IC),1 which translocates to the nucleus.

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1 The abbreviations used are: IC, intracellular domain; E1, ubiquitin-activating enzyme; E2, ubiquitin carrier protein; E3, ubiquitin-protein isopeptide ligase; HA, hemagglutinin; GST, glutathione S-transferase; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; GAL4DB, GAL4 DNA binding domain; UAS, upstream activating sequence.

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4 The Caenorhabditis elegans sel-10 protein contains an F-box and WD40 repeats (10), which structurally similar to E3 ubiquitin ligases has led to the proposal that sel-10 negatively regulates Notch activity (10). Furthermore, sel-10 acts at the level of the intracellular domain, since loss-of-function of sel-10 reduces the effects of a lin-12 intracellular domain (10). The sel-10 protein contains an F-box and WD40 repeats (10), which are characteristic of E3 ubiquitin ligases. E3 ubiquitin ligases are, together with the E1 ubiquitin-activating enzyme and E2 ubiquitin-conjugating enzymes, responsible for targeting proteins for proteasome-mediated protein degradation (11, 12). E3 ubiquitin ligases attach ubiquitin to target proteins, thereby allowing them to be recognized and degraded by the 26 S proteasome. There are a large number of E3 ubiquitin ligases, each with specificity for particular target proteins. The fact that sel-10 negatively regulates Notch and presenilins and is structurally similar to E3 ubiquitin ligases has led to the proposal that sel-10 may act by targeting Notch and presenilins for proteasome-mediated degradation (10).

In this report, we characterize a mammalian homolog of Sel-10 (mSel-10) and show that mSel-10 ubiquitinates Notch 1 IC and negatively regulates Notch signaling. We also demonstrate that mSel-10 itself is ubiquitinated and subject to proteasome-mediated degradation. This shows that ubiquitination and proteasome-mediated degradation are important control mechanisms for Notch activity, and it defines a role for mSel-10 in this process.

In the nucleus, the Notch IC binds to the DNA-binding protein CSL (RBP-Jk) and functions as a transactivating protein, controlling the expression of Notch target genes. The most well characterized target genes are the HES genes, which encode negative transcriptional regulators of the basic helix-loop-helix type (for review, see Ref. 7). The Notch receptor is thus both the sensor of the ligand and the provider of a transactivating domain for regulation of downstream genes. A potential problem associated with using this mode of signaling is that the signal is not controlled by the ligand once the Notch IC is cleaved. Thus, a long-lived Notch IC would keep the cell in a prolonged and uncontrolled “on-state,” in particular since only very small amounts of Notch IC are required for signaling (8). It therefore seems reasonable that the activity of the intracellular domain is under strict control, either by Notch-modulating proteins that control its activity (7), or by rapid turnover and proteolysis of the intracellular domain.

To gain further insights into the control and degradation of the mammalian Notch IC, we have focused our interest on the gene for Sel-10. Work in the nematode Caenorhabditis elegans demonstrates that the gene for sel-10 is a negative regulator of Notch. sel-10 was originally identified by Sundaram and Greenwald (9) in C. elegans through a genetic screen for modifiers of the Notch homolog lin-12. Loss of sel-10 activity rescues a lin-12 hypomorph, suggesting that sel-10 negatively regulates Notch activity (10). Furthermore, sel-10 acts at the level of the intracellular domain, since loss-of-function of sel-10 reduces the effects of a lin-12 intracellular domain (10). The sel-10 protein contains an F-box and WD40 repeats (10), which are characteristic of E3 ubiquitin ligases. E3 ubiquitin ligases are, together with the E1 ubiquitin-activating enzyme and E2 ubiquitin-conjugating enzymes, responsible for targeting proteins for proteasome-mediated protein degradation (11, 12). E3 ubiquitin ligases attach ubiquitin to target proteins, thereby allowing them to be recognized and degraded by the 26 S proteasome. There are a large number of E3 ubiquitin ligases, each with specificity for particular target proteins. The fact that sel-10 negatively regulates Notch and presenilins and is structurally similar to E3 ubiquitin ligases has led to the proposal that sel-10 itself is ubiquitinated and subject to proteasome-mediated degradation. This shows that ubiquitination and proteasome-mediated degradation are important control mechanisms for Notch activity, and it defines a role for mSel-10 in this process.
Experimental Procedures

DNA Constructs and Cloning—Sel-10-Myc is the cDNA encoding the entire human Sel-10 in the pCS2 vector. Sel-10ΔWD40 was constructed by partial cleavage of Sel-10-Myc with NcoI followed by blunting and religation. For green fluorescent protein-Sel-10, the entire human Sel-10 cDNA subcloned in frame into pEGFP-C3 (CLONTECH, Palo Alto, CA). Sel-10ΔMyc was constructed through insertion of EcoRI- and HindIII-cleaved Sel-10 into EcoRI- and HindIII-cleaved pCMX-PL1. Notch 3 IC, GAL4DB-Notch 1 IC, GST-1111, and GST-3333 have been described previously (see below). For GST-1110, GST-1111 was cleaved...
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**RESULTS**

A Mammalian Sel-10 Homolog—To characterize the mammalian sel-10 homolog, cDNA expressed sequence tag clones for mouse and human Sel-10 were obtained through database searches with the C. elegans sel-10 sequence, and extended by rapid amplification of cDNA ends (GenBank® accession no. AY008274). The mouse and human homologs are nearly identical (98% identity at the amino acid level) and share the principal structure with the C. elegans sel-10 gene (Fig. 1A). The F-box and the seven WD40 repeats are highly conserved.

**Fig. 3.** mSel-10 reduces transcriptional activation specific promoters in a presenilin-independent fashion. **A**, co-transfection of mSel-10 (100 ng) and Notch 1 IC (100 ng) in COS-7 cells. mSel-10 reduces the Notch 1 IC-mediated activation of the HES 1 promoter luciferase reporter construct (200 ng transfected), whereas a mSel-10 construct lacking the WD40 repeats results in a much smaller reduction (not statistically significant). B, co-transfection of mSel-10 and Notch 1 IC together with a HES 1 reporter construct in B95 cells, which are deficient for presenilin 1 and 2. mSel-10 reduces the activity of the Notch construct also in the absence of presenilin. C, the activity of a GAL4DB-Notch 1 IC fusion protein on the UAS-luciferase reporter gene is reduced in the presence of mSel-10. ***p < 0.0001 versus Notch 1 IC. ***, p < 0.001 versus GAL4DB-Notch 1 IC. D, the activity of GAL4DB/VIP16TA on the UAS-luciferase reporter gene is not significantly reduced in the presence of mSel-10. For statistics, we used the analysis of variance factorial t test (p < 0.1) (StatView, Cary, NC). The data represent the mean of three or more independent experiments performed in triplicate.

with XhoI followed by blunting and religation. For GST-3330, GST-3333 was partially cleaved with SacII, gel-purified, and cleaved with NotI followed by blunting and religation. For GST-3300, GST-3333 was cleaved with SmaI, gel-purified, and cleaved with NotI followed by blunting and religation. Notch 1 IC-6-Myc was constructed by digesting Notch 1 IC in pBluescript with BamHI, followed by blunting and digestion with SalI. This fragment was inserted into the pCS2 vector cleaved with EcoRI, blunted, and cleaved with XhoI. Notch 1EΔ has been described previously (8), and so has the HA-tagged ubiquitin construct (13).

GST Pull-down Assays—Escherichia coli BL21 was transformed with p6EX-4T-3, GST-Notch 1 IC (GST-1111), GST-Notch 3 IC (GST-3333) (14), or deletion constructs fused to GST (see above). The fusion proteins were induced and extracted according to the manufacturer’s instructions (Amersham Pharmacia Biotech, Uppsala, Sweden). 35S-Labeled Sel-10 protein was synthesized using the TNT-coupled rabbit reticulocyte lysate system (Promega, Madison, WI). 35S-Labeled Sel-10 was mixed with the various GST fusion proteins and incubated for 1 h at 4 °C in 200 μl of G-buffer (20 mM Tris-HCl, pH 7.9, 150 mM KCl, 1 mM EDTA, 4 mM MgCl2, 0.2% Nonidet P-40, and 10% glycerol). Fusion proteins were washed six times in G-buffer, and bound protein was released in sample buffer (2% SDS, 10% glycerol, 80 mM Tris, pH 6.8, 0.72 M β-mercaptoethanol) and analyzed by 9% SDS-PAGE. Transfections and Reporter Gene Analysis—Monkey kidney COS-7 cells were transiently transfected using the LipofectAMINE Plus reagent (Life Technologies, Inc.) according to the manufacturer’s instructions. Each transfection contained 50 ng of CMV-LacZ plasmid, 200 ng of reporter plasmid (HES-1 or MH100-tk-luc), and various amounts of different expression plasmids and mock plasmid to a total of 450 ng. The cells were harvested 36–48 h after transfection in 150 μl of lysis buffer. Luciferase activity was assayed using GeneGlow (Biothea, Dalarö, Sweden) and measured in an Anthos Luminoscan Lucy 1. Transfection efficiency was determined by measuring β-galactosidase activity in the cell lysate.

Immunocytochemistry—C2C12 cells were plated on glass slides in 24-well plates and transfected (see above) with 200 ng of Sel-10-Myc and Notch 1 IC-HA expression plasmids. Immunostaining was performed using a rabbit anti-c-Myc 1:50 (CLONTECH, Palo Alto, CA) and mouse anti-HA antibody 12CA5 1:500 (Berkeley Antibodies Inc., Richmond, CA). Secondary antibodies were Alexa rabbit 488 and Alexa mouse 546 (Molecular Probes Inc., Eugene, OR). Immunoreactivity was visualized by fluorescence microscopy and photographed, and the pictures were assembled using PhotoShop (Adobe Systems Inc., Mountain View, CA).

Immunoprecipitation—Cells transfected with either 4 μg of pcMX vector or 2 μg of Notch 1 IC-6-Myc, Sel-10-Myc, and Notch 1EΔ together with 1 μg of Ub-HA were washed once in PBS, trypsinized, and centrifuged, and the cell pellet was washed in PBS. After addition of 300 μl of lysis buffer (1% SDS, 50 mM Tris, 100 mM NaCl), the samples were incubated on ice for 5 min followed by addition of 1200 μl of TN-buffer (50 mM Tris, 100 mM NaCl, 0.5% sodium deoxycholate, 1% Triton X-100, 1% bovine serum albumin) and a 10-min incubation on ice. The genomic DNA was discarded, and 4 μl of mouse anti-c-Myc antibody 9E10 (PharMingen, San Diego, CA) was added. After 1 h at 4 °C, 50 μl of Sepharose G (Amersham Pharmacia Biotech) was added and the samples were incubated for another 1 h at 4 °C. The samples were then washed four times with 800 μl of TN-buffer, followed by SDS-PAGE and Western blot analysis.

Western Blot—The proteins were subjected to 8–11% SDS-PAGE, blotted to a nitrocellulose membrane, and probed with the indicated antibodies. The membrane was washed extensively in PBS with 0.05% Tween 20. Antibody concentrations were as follows: anti-HA (12CA5), 1:1000; anti-c-Myc (9E10), 1:250; and rabbit anti-ubiquitin Z0458 (DAKOPATTS, Stockholm, Sweden), 1:50. Where indicated the membrane was stripped at 56 °C for 40 min in Strip buffer (100 mM β-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl).
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To demonstrate that Notch IC and mSel-10 physically interact, we showed that Notch 1 IC interacted specifically with 35S-labeled in vitro translated mSel-10 in a GST pull-down experiment (Fig. 2f). To map more precisely which part of Notch IC is responsible for the interaction, we tested deletion mutants of Notch 1 IC for binding to mSel-10. Binding of mSel-10 occurs in the RAM, ankyrin repeat, or RE/AC domains (for nomenclature, see Ref. 15), since a construct lacking the COOH-terminal region (N1ΔC-term) using the Myc antibody 9E10 and detection by Western blot using the same antibody. N1ΔC-term can be immunoprecipitated, both in the presence and absence of mSel-10 and MG132. D, after stripping, the membrane was reprobed with an ubiquitin antibody. No ubiquitin immunoreactivity can be observed for N1ΔC-term.

To learn whether the mouse genome contains genes highly related to mSel-10, we analyzed genomic mouse DNA for the presence of related sequences by Southern blot analysis, using a probe from the highly conserved WD40 repeat region (Fig. 1D). Only the two bands predicted from sequencing across the genomic mouse sel-10 locus (data not shown) were observed on the Southern blot, at both high and low hybridization stringency (Fig. 1D). In keeping with this, we did not find additional highly conserved members in data bases of the human or mouse genomes (data not shown). We thus conclude that we have identified the mammalian Sel-10 homolog, and that it retains the cardinal features of the C. elegans gene, i.e. an F-box and WD40 repeats.

Mammalian Sel-10 and Notch IC Physically Interact and Are Predominantly Localized to the Nucleus—In C. elegans, sel-10 has been shown to physically interact with mouse Notch 4 IC and loss of sel-10 function leads to increased lin-12 signaling (10). Since sel-10 presumably regulates Notch signaling at the level of the Notch IC (10), we first wanted to learn whether Notch IC and mSel-10 are localized to the same subcellular compartment. Analysis of the intracellular distribution of immunotagged versions of Notch 3 IC and mSel-10 by immunochemistry demonstrates that Notch 3 IC and mSel-10 are predominantly localized to the nucleus and only small amounts are found in the cytoplasm (Fig. 2A-F). No specific signal was observed when the primary antibodies were omitted (Fig. 2G and H).

To map more precisely which part of Notch IC is responsible for the interaction, we tested deletion mutants of Notch 1 IC for binding to mSel-10. Binding of mSel-10 occurs in the RAM, ankyrin repeat, or RE/AC domains (for nomenclature, see Ref. 15), since a construct lacking the COOH-terminal region (N1ΔC-term) using the Myc antibody 9E10 and detection by Western blot using the same antibody. N1ΔC-term can be immunoprecipitated, both in the presence and absence of mSel-10 and MG132. D, after stripping, the membrane was reprobed with an ubiquitin antibody. No ubiquitin immunoreactivity can be observed for N1ΔC-term.

Through searches in the Drosophila data base, we also identified a Drosophila sel-10 homolog (dSel-10) (Flybase accession no. FBgn0035516). dSel-10 shows 78% amino acid identity to human Sel-10 and 60% to C. elegans sel-10; the F-box and the WD40 repeats are the most highly conserved domains (Fig. 1, A-C).

Fig. 4. Notch 1 IC is ubiquitinated by mSel-10 and ubiquitination requires the COOH-terminal region in Notch 1 IC. A, transfection of COS-7 cells with the Notch 1 IC (2 μg) together with HA-tagged ubiquitin (1 μg) in all experiments and mSel-10 (1 μg) where indicated. Panel shows immunoprecipitation using the Myc antibody 9E10 and detection by Western blot using the same antibody. B, after stripping, the membrane was reprobed with an ubiquitin antibody. Note the ubiquitin immunoreactivity observed in the presence of MG132 (concentration 3 μM overnight) and mSel-10 (lane 6). C, immunoprecipitation of a construct lacking the COOH-terminal region of Notch 1 IC (N1ΔC-term) using the Myc antibody 9E10 and detection by Western blot using the same antibody. N1ΔC-term can be immunoprecipitated, both in the presence and absence of mSel-10 and MG132. D, after stripping, the membrane was reprobed with an ubiquitin antibody. No ubiquitin immunoreactivity can be observed for N1ΔC-term.
Sel-10 Reduces Notch IC-GAL4 Activation through an RBP-Jk-independent Promoter—The reduction of Notch 1 IC-mediated activation from the HES 1 promoter by mSel-10 (Fig. 3A) could be the result of interference with Notch IC or with RBP-Jk. To distinguish between these two possibilities, we studied the effect of mSel-10 on Notch IC activation in an RBP-Jk-independent promoter context. We analyzed whether the presence of mSel-10 would reduce the activity of a Notch 1 IC-GAL4 DNA binding domain fusion protein (GAL4DB-Notch 1 IC) on the UAS promoter. In agreement with previous results (14, 15), transfection of only GAL4DB-Notch 1 IC resulted in strong activation, but this was reduced by ~50% through the addition of mSel-10 (Fig. 3C). As a control, the effect of mSel-10 on GAL4 DB/VP16 TA was measured, and although there was a slight decrease after addition of mSel-10, this reduction was not statistically significant (Fig. 3D).

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MG132 leads to increased levels of Notch 1 IC but reduced activation of a HES promoter. **A**, the activity of extracts of transfected Notch 1 IC on a HES 1-luciferase reporter gene in the absence and presence of MG132. Note that activity is reduced when MG132 is added. **B**, a Western blot of Notch 1 IC protein levels from the same extracts used to generate the activity data in A, visualized by a Myc antibody. Note the strong increase in the amount of Notch 1 IC in the presence of MG132. Error bars are standard deviation from three parallel transfections (pooled to give the Western blot data).

**DISCUSSION**

In this report, we describe the characterization of the mammalian Sel-10 gene and its regulation of Notch signaling. We find that the mouse and human genomes contain one highly

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**Table: Levels of Notch 1 IC Protein**

| Condition | Notch 1 IC | pCMX |
|-----------|------------|------|
| Absence   | -          | +    |
| Presence  | +          | -    |

**Figure 5**

MG132 leads to increased levels of Notch 1 IC but reduced activation of a HES promoter. **A**, the activity of extracts of transfected Notch 1 IC on a HES 1-luciferase reporter gene in the absence and presence of MG132. Note that activity is reduced when MG132 is added. **B**, a Western blot of Notch 1 IC protein levels from the same extracts used to generate the activity data in A, visualized by a Myc antibody. Note the strong increase in the amount of Notch 1 IC in the presence of MG132. Error bars are standard deviation from three parallel transfections (pooled to give the Western blot data).
conserved sel-10 homolog, and that this is true also for Drosophila. When the sel-10 sequences from C. elegans, Drosophila, and man are compared, some of the most highly conserved regions are the F-box and WD40 repeats, in agreement with a role for mSel-10 as an E3 ubiquitin ligase mSel-10 is predominantly localized to the nucleus and reduces the Notch IC-mediated transcriptional activation from a HES promoter in a presenilin-independent manner. The observed reduction of HES promoter activation at the level of the Notch intracellular domain agrees with genetic findings from C. elegans, where loss of sel-10 rescues a lin-12 (Notch) hypomorph and leads to augmentation of a phenotype caused by overexpression of the lin-12 intracellular domain (10). No role has yet been ascribed to the Drosophila sel-10 homolog, but mutations in the 26 S proteasome subunit in Drosophila lead to increased Notch IC levels (17), indicating that there is a link between Notch IC levels and proteasome function also in Drosophila.

We demonstrate that Notch IC is ubiquitinated by mSel-10. Ubiquitination of Notch 1 IC requires the 400 most carboxyl-terminal amino acid residues in Notch 1 IC. Interestingly, this region contains a PEST domain, and PEST domains have in a number of other proteins been shown to play a role in the degradation of the protein (for review, see Ref. 11). Since the PEST domain is highly conserved in all Notch ICs, and thus likely to be important for function, it is possible that a role for the Notch IC PEST domain is to control mSel-10-mediated ubiquitination-dependent turnover of the Notch IC domain. In addition to mSel-10 one other E3 ubiquitin ligase, Itch, has been shown to ubiquitinate Notch IC (18). Interestingly, Itch-mediated ubiquitination of Notch 1 IC in T cells appears not to require the PEST domain (18).

It is noteworthy that ubiquitination of Notch 1 IC by mSel-10 is only observed in the presence of the proteasome inhibitor MG132. This suggests that Notch 1 IC ubiquitinated by mSel-10 is normally rapidly degraded by the proteasome, and therefore does not accumulate in cells where proteasome function is not blocked. It was therefore somewhat unexpected that the total amount of Notch 1 IC was not reduced in cells expressing transfected mSel-10, but this may be a consequence of the large amounts of Notch 1 IC produced after transfection, which may mask a small decrease caused by the increased mSel-10 activity. The finding that Notch 1 IC is ubiquitinated and subjected to proteasome-mediated degradation suggests that the Notch IC is turned over faster than the reminder of the Notch receptor. A rapid turnover of the Notch IC would quickly extinguish the transactivation signal in the nucleus if no more Notch receptor was cleaved at the cell surface. The ubiquitination-mediated inactivation of Notch IC signaling may in fact not exclusively depend on the degradation of the Notch IC by the proteasome. The observation that, in the presence of MG132, the levels of Notch 1 IC protein increase at the same time as HES activation is reduced (Fig. 5).
may argue that ubiquitinated Notch 1 IC is a less potent transactivator, which would lead to attenuation of Notch signaling already at the stage of Notch IC ubiquitination, i.e. before Notch IC is degraded. We do not yet know whether ubiquitination affects binding of Notch 1 IC to the DNA-binding protein CSL or whether it masks a transactivation domain in Notch 1 IC.

We found that mSel-10 protein levels were dramatically increased in the presence of MG132, and that immunoprecipitated mSel-10 was ubiquitinated specifically after addition of MG132. This demonstrates that mSel-10 itself is a target for ubiquitination and proteasome-mediated degradation. It is interesting to observe that the E3 ubiquitin ligase cdc4p, which is the protein structurally most closely related to mSel-10 (10, 16), recently was found to be subject to ubiquitination and degradation (20). In the case of cdc4p, ubiquitination appears to be carried out by an auto-ubiquitination mechanism (20), but whether this is the case also for mSel-10 remains to be established.

In addition to the role in Notch IC ubiquitination demonstrated here, mSel-10 also functions as an E3 ubiquitin ligase in its interaction with its other target protein, presenilin. Physical interaction between mSel-10 and presenilin 1 is observed only in the presence of proteasome inhibitors and presenilin is ubiquitinated by mammalian Sel-10. This observation, combined with the findings presented here and the fact that presenilins are required for the proteolytic processing of Notch receptors (21–23), reveals a complex interplay between mSel-10, Notch, and presenilin, where ubiquitination and proteasome-mediated degradation play a pivotal role. This is outlined in a hypothetical model in Fig. 7. Briefly, the levels of mSel-10 are under control of the proteasome in an ubiquitin-dependent manner, and mSel-10 negatively regulates the activity of both Notch IC and presenilins, also in an ubiquitination-dependent fashion. In this model, increased proteasome activity would initially reduce Notch and presenilin activity, but may also lead to a reduction of mSel-10, which could allow Notch and presenilin signaling to increase again. Conversely, under conditions of reduced proteasome activity, more mSel-10 accumulates, which may contribute to the accumulation of ubiquitinated Notch IC, accompanied by the observed reduced HES activation. Furthermore, as presenilins are positive regulators of the intracellular cleavage of the Notch receptor (21–23), an interesting consequence of the model in Fig. 7 is that mSel-10 could negatively regulate Notch activity in two separate ways: both by its direct action on Notch IC and by reducing cleavage of Notch as a consequence of reducing presenilin levels. In conclusion, the data presented here demonstrate that ubiquitination and proteasome-mediated degradation are important mechanisms for controlling activity and turnover of Notch ICs, and that mSel-10 plays a key role in this process.

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Addendum—During the course of this work, another report demonstrating that mSel-10 negatively regulates Notch IC was published by Gupta-Rossi et al. (Gupta-Rossi, N., Le Bail, O., Gonén, H., Brou, C., Logeat, F., Six, E., Ciechanover, A., and Israel, A. (2001) J. Biol. Chem. 276, published on June 25, 2001).

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