INTRODUCTION

Stress conditions in the endoplasmic reticulum (ER) cause the accumulation of misfolded proteins in the ER lumen or membrane. The unfolded-protein response (UPR) is a collection of signaling pathways that regulate the adaptation of cells to ER stress (Schroder and Kaufman, 2005; Ron and Walter, 2007). The general effects of the UPR are a decrease in protein synthesis and translocation to the ER, an increase in retrotranslocation and degradation of misfolded ER-localized proteins by the ER-associated degradation (ERAD) machinery, and an increase in the volume of the ER and in its protein-folding capacity. Severe ER stress can lead to autophagy and also to cell death (Schroder and Kaufman, 2005; Zhang and Kaufman, 2006; Ron and Walter, 2007). The UPR in mammals includes three stress sensors: inositol requirement 1 (IRE1), protein kinase-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) (Schroder and Kaufman, 2005). The UPR is activated not only by external stress but also during development and cellular differentiation, when changes in the physiological state of the cell cause different levels of protein-folding load upon the ER (Zhang and Kaufman, 2006). The UPR in Caenorhabditis elegans is regulated by orthologues of IRE1, PERK and ATF6 (IRE-1, PEK-1 and ATF-6, respectively) and mediates the expression of multiple genes under both normal and ER-stress conditions (Shen et al., 2001; Shen et al., 2005).

The ERAD pathway complements the activity of the UPR molecular chaperones and modifying enzymes in quality control by removing misfolded secretory and membrane proteins, as well as unassembled subunits of multimeric proteins from the ER. The ERAD process starts with the recognition of a substrate in the ER, retrotranslocation from the ER lumen to the cytoplasm through a channel (Meusser et al., 2005; Romisch, 2005; Vembar and Brodsky, 2008), and ubiquitination through a cascade of enzymatic reactions by using ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) (Hershko et al., 2000). The polyubiquitin chain is recognized by the CDC48/p97 ATPase complex (CDC48/p97-Ufd1-Npl4) that transports substrates to the proteasome (Vembar and Brodsky, 2008).

E3 ubiquitin ligases in ERAD function as quality-control ligases, recognizing misfolded proteins and subunits that lack their oligomerization partner (Kostova et al., 2007). In yeast, Doa10p and Hrd1p are membrane-associated RING finger ligases that have been found to degrade all studied yeast ERAD substrates (Vashist and Ng, 2004; Carvalho et al., 2006; Denic et al., 2006). Orthologues of Doa10p and Hrd1p have been identified in higher organisms, as have additional ERAD-related cytoplasmic and ER-membrane-bound E3 ligases (Kostova et al., 2007) whose specific in vivo targets and functions are largely unknown. The main mammalian membrane-bound E3 ligases related to ERAD are Hrd1/synoviolin, gp78, TEB4/MARCHVI, and RNF5 (Fang et al., 2001; Amano et al., 2003; Nadav et al., 2003; Kikkert et al., 2004; Chen et al., 2006; Kreft et al., 2006; Omura et al., 2006; Tsai et al., 2007). The C. elegans genome encodes orthologues of Hrd1, gp78, MARCHVI, and RNF5 (named HRD-1, HRDL-1, MARC-6, and RNF-5, respectively) (Sasagawa et al., 2003; Kikkert et al., 2004). Depletion of HRD-1 or MARC-6 causes increased sensitivity of worms to ER stress (Sasagawa et al., 2007). RNF5/RMA1 is a membrane-anchored RING finger ligase that affects the localization and levels of LIM-domain-containing protein in C. elegans and of paxillin in mammalian cells (Matsuda and Nakano, 1998; Matsuda et al., 2001; Didier et al., 2003; Brodsky et al., 2004, 2007) and has been implicated in ERAD (Younger et al., 2006; DeLaunay et al., 2008; Tcherpakov et al., 2009). In C. elegans, the ...
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numerical aperture objective lens. Double transgenic worms were recorded using multitracking configuration to prevent signal cross-talk. RNF-121::GFP/mRFP::SP12 (1.1 scan zoom, pinhole 1.4 Airy Unit, 83.2 × 22.3 μm confocal image size) and RNF-121::GFP; mRFP::MANS (2.0 scan zoom, pinhole 1.3 Airy Unit, 47.8 × 14.8 μm confocal image size). Worms were mounted on 4% agarose pads and anesthetized with 0.1% tricaine and 0.01% tetramisole. Mean fluorescence intensities were quantified using LSM 5 EXCITER software. The effect of induced RNF-121 on PAT-3-GFP expression in the body wall muscle was weaker; therefore, quantification was done by normalization of the fluorescence intensity in the distal tip cells (DTC) to the fluorescence intensity in the muscle perinuclear region. For fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLP) analyses, laser power and gain were adjusted so that pixels in the inclusions were under saturation levels. For FRAP experiments, photobleaching was performed by 4× iterative scanning (100% transmission) focused on a region of 1 μm². Images were acquired (1% transmission) in a single Z-plane using open pinhole at 10-s intervals. In FLP experiments, a region outside an inclusion was repeatedly bleached 1× scanning) at 10-s intervals, and images were acquired after each bleach.

RESULTS

RNF-121 Is Localized to the ER in C. elegans and Exhibits In Vitro E3 Ubiquitin Ligase Activity

RNF-121 (C16C10.5) is a highly conserved protein with putative transmembrane domains (TMDs) and a RING finger domain (C3H2C3 type) (Figure 1A and Supplementary Table S1). These results suggested that inactivation of RNF-121 induces a low level of ER stress that does not interfere with normal development under optimal growth conditions but has an inhibitory effect on development in response to relatively low levels of exogenous ER stress. Next, we tested whether reduced RNF-121 activity elicits the UPR pathway. The proteins HSP-3 and HSP-4 are the C. elegans orthologues of the mammalian Grp78/BIP (Shen et al., 2001). We analyzed the expression levels of the hsp-4::gfp transcriptional reporter (Calfon et al., 2002) in rnf-121(RNAi)-treated worms. In control wild-type worms, the hsp-4::gfp reporter displays low fluorescence levels, whereas the expression is strongly induced in the epidermis and intestine upon UPR activation (Calfon et al., 2002). The rnf-121(RNAi)-treated worms exhibit high levels of intestinal GFP expression under normal growth conditions (Figure 2B, bottom, arrow). Higher hsp-4::gfp levels were also observed in the rnf-121(ok848) mutant worms (Supplemental Figure S2). This increase in hsp-4::gfp expression demonstrated that reducing the level of RNF-121 causes UPR activation.

RNF-121 Acts through the PERK/PEK-1 Signaling Pathway

The above-mentioned experiments indicate that depletion of RNF-121 causes ER stress and activates the UPR. To determine whether RNF-121 acts via the UPR pathway, we analyzed sensitivity to ER stress after depletion of RNF-121 in a mutant background of each of the three known ER stress transducers in C. elegans: IRE-1, PEK-1, and ATF-6. The ire-1(ok33) and pek-1(ok275) mutant strains are more sensitive to tunicamycin than the wild-type (Figure 3A, – bars; Shen et al., 2001), whereas atf-6(ok551) worms are less sensitive to tunicamycin (Shen et al., 2005), and in our hands are more resistant than the wild type (Figure 3A, – bars). Sensitivity to tunicamycin was increased in ire-1(ok33) rnf-121(RNAi), and atf-6(ok551); rnf-121(RNAi) compared with the ire-1(ok33) and atf-6(ok551) mutant worms, respectively (Figure 3A, compare – to + bars, and Supplemental Tables S1–S4; p < 0.005). However, rnf-121(RNAi) treatment had no significant effect on tunicamycin sensitivity of pek-1(ok275) mutant worms (p > 0.2) (Figure 3A, bottom, 2–3 μg/ml tunicamycin-
cin, and Supplemental Table S5). These results suggest that RNF-121 operates in *C. elegans* in the same genetic pathway with PEK-1. Because *pek-1(ok275)* worms are more sensitive to tunicamycin than *rnf-121(RNAi)*-treated wild-type N2 worms (Figure 3A and Supplemental Tables S1 and S5), this suggests that *rnf-121* is located downstream of *pek-1* and therefore depletion of *rnf-121* did not cause a more severe phenotype in the *pek-1(ok275)* mutant worms. PERK not only mediates general translational attenuation upon ER stress but also increases the translation of the ATF4 transcriptional activator and thus regulates gene expression during ER stress response (Scheuner et al., 2001; Harding et al., 2003). To determine whether the transcription of *rnf-121* is regulated by PEK-1 and the UPR pathway in *C. elegans*, we performed a real-time PCR analysis of the mutant strains *pek-1(ok275)*, *ire-1(v33)*, and *atf-6(ok551)*, as well as of wild-type worms, treated with the UPR inducers DTT, tunicamycin, and thapsigargin. Although the mRNA levels of *hsp-4* were induced upon tunicamycin or DTT treatment and in *pek-1(ok275)* and *atf-6(ok551)* mutant backgrounds, and abolished in *ire-1(e33)*.
as shown previously (Shen et al., 2001), the levels of rnf-121 mRNA were largely unaffected (Figure 3B). No changes in rnf-121 mRNA levels were detected when any of the UPR mutants were treated with tunicamycin (data not shown). These results indicate that RNF-121 is not regulated on the transcriptional level by PEK-1 and the UPR system. To examine whether RNF-121 protein level is regulated by the PEK-1 pathway, we performed immunoblot analysis of RNF-121::GFP worms treated with tunicamycin and DTT. Although the level of RNF-121 increases after ER stress in wild-type background, it is constantly high in pek-1(ok275) worms (Figure 3C) and does not increase after ER stress. It suggests that PEK-1 pathway activates RNF-121 translation or protein stability under ER stress conditions, whereas inactivation of PEK-1 during development in the pek-1(ok275) mutant may cause ER stress and up-regulation of RNF-121 by an alternative pathway.

**Overexpression of RNF-121 Causes Defects in Gonad and Germline Development That Depends on DER-1/Derlin-1**

The number of identified ER-membrane located E3-ligases in multicellular organisms is increasing, suggesting specific roles for these E3s as the ability to recognize specific ERAD substrates. We expressed RNF-121 at specific stages of development by using a heat-inducible promoter. Moderate decrease in worm motility was detected following RNF-121 overexpression in adults (Supplemental Figure S3). We observed defects in normal development only when RNF-121 was induced at a specific developmental stage, the mid-L2 stage, resulting in decreased hermaphrodite fertility. The average number of progeny of hsp-16p::RNF-121 was 55 ± 18 (n = 65) compared with 254 ± 28 (n = 60) in wild-type worms treated with the same heat-shock conditions at the mid-L2 stage (Supplemental Figure S4). To characterize this partial sterility phenotype, we analyzed gonad morphology and oocyte development, maturation, and ovulation in young adult worms. The C. elegans gonad is formed from two symmetrical U-shaped arms, each connected by a spermathea to a common uterus. At the L2 stage, migration of the hermaphrodite gonads begins, guided by two special somatic cells, the DTCs (Kimble and White, 1981). Germ cells proliferate in the distal region of the gonad and as they move proximally, they enter meiosis and differentiate into sperm or oocyte. Oocyte meiotic maturation occurs at the most proximal gonad, followed by entrance into the spermathea at ovulation and fertilization (McCarver et al., 1999). After induction of RNF-121 at the mid-L2 stage, the adult gonad arms exhibited defective gametogenesis that resulted in proximal gonads with a small number of oocytes (Figure 4, C–H, 3.6 ± 1.5 oocytes per gonad arm, n = 61) in mid- and late-stage adults compared with 9.6 ± 1.5 oocytes in mid-stage wild-type adults (n = 49) and 9.2 ± 1.3 in mid-stage adults expressing a ligase-inactive RNF-121 mutant (hsp-16::RNF-121C222G/C225A, n = 67). DIC and 4,6-diamidino-2-phenylindole (DAPI) analyses revealed delayed development of the oocytes in the proximal gonads (Figure 4, C–E, compared with Figure 4, A and B). In wild-type gonad, progression of nuclei from pachytene into diplotene occurs in the loop region, whereas in the proximal gonad arm oocytes progress to diakinesis (Figure 4B, short arrow). In contrast, pachytene nuclei were found in the hsp-16p::RNF-121 proximal gonads after the loop region, indicating a delay in progression into diplotene (72.6 ± 4.5%; n = 256) (Figure 4E, arrowhead). In addition, the developing oocytes and especially the most proximal oocyte had abnormal elongated shapes (Figure 4, C, D, and F, white outline) and were not rectangular as the wild-type oocytes before maturation (Figure 4A, white outline) (McCarver et al., 1999). Meiotic maturation rates were slower (0.85 ± 0.23 maturations/gonad arm/h; n = 27) relative to the maturation rates in wild-type hermaphrodite that were treated with the same heat-shock conditions (2.65 ± 0.50 maturations/gonad arm/h; n = 16). Abnormal signaling from the sperm to the oocyte or sheath cells that surround the oocyte could
contribute to the lower maturation phenotype (McCarter et al., 1999; Miller et al., 2001). Because the sperm in the hsp-16::RNF-121 adults seem abnormal and swollen (Figure 4F, arrow), we examined whether cross to wild-type males will rescue the partial sterility phenotype. Indeed, the wild-type sperm partially rescued the sterility and the number of progeny increased (118 ± 37, n = 31) (Supplemental Figure S4).

In addition to abnormalities in oocyte development and maturation after induction of RNF-121, we observed gonad migration defects that involved extra turns and abnormal migration paths, as a 45° migration toward the dorsal side followed by a reversed migration to the ventral side and repeated migration toward the dorsal side (Figure 4G) or a reversed migration on the ventral side till the midbody (Figure 4H). These phenotypes depend on the expression of
an intact RING finger domain of RNF-121 and could be detected at lower percentage without heat shock treatment, suggesting that low levels of wild-type protein expressed through the basal activity of the heat shock promoter are effective, in contrast to a RING mutant (Figure 4I). To determine the functional relation between the E3 ligase activity of RNF-121 and the ERAD pathway, we induced RNF-121 expression after depletion of the putative retrotranslocation component der-1 (Ye et al., 2004). Depletion of der-1 in wild-type background had no effect on gonad development (Figure 4I). However, analysis of gonads in der-1(RNAi) adult hermaphrodites after induction of RNF-121 suppressed the RNF-121 effect and showed low penetrance of the gonad phenotype (Figure 4I), suggesting that RNF-121 acts with and requires Derlin-1 for its activity in the ERAD pathway. We conclude that ectopic expression of RNF-121 at early stage of gonad development impaired gonad and germline development and that this activity depends on DER-1, probably by contributing to active retrotranslocation of substrates from the ER to the cytosol.

**PAT-3/β-Integrin::GFP Is a Substrate for RNF-121**

The *C. elegans* integrin receptors are necessary for normal gonad development and their depletion results in similar phenotypes as described above after overexpression of RNF-121. In *C. elegans*, there are two α-integrin subunits, INA-1 and PAT-2, and one β-integrin subunit, PAT-3 (Cox et al., 2004). PAT-3 is expressed in the body-wall muscles and the somatic gonad (Hresko et al., 1994; Gettner et al., 1995) and is essential for muscle attachment and organization of the sarcomere during embryogenesis (Williams and Waterston, 1994) and for gonad migration and oocyte development and maturation during postembryonic development (Lee et al., 2001, 2005). In mammalian cells, integrin-β1 is expressed in excess over integrin α-chain, and the excess of noncomplexed β1 is retained in the ER and degraded (Heino et al., 1989; Koivisto et al., 1994). To check whether PAT-3 is an ERAD substrate for RNF-121 E3 ligase activity in vivo, we used the PAT-3/β-integrin::GFP transgenic strain (Plenefisch et al., 2000). The GFP-tagged β subunit expressed through the PAT-3::GFP reporter exhibits a similar pattern as the anti-PAT-3 (MH25) antibody (Francis and Waterston, 1991) with an additional ER membrane localization, probably as a result of high expression levels of the β subunit (Figure 5A). This suggests that the unassembled β subunits are retained in the ER and subjected to degradation through the ERAD pathway. To test the possibility that PAT-3::GFP is degraded by RNF-121, we first measured GFP levels in the

**Figure 4.** Overexpression of RNF-121 at the L2 stage results in germline and gonad defects. (A and B) DIC and DAPI staining of wild-type adult hermaphrodite gonad arms. Marked are pachytene nuclei (arrowhead), diplotene (long arrow), and oocytes at diakinesis (short arrow). A scheme of the DTC path is shown on the left. (C–E) DIC and DAPI staining of hsp-16p::RNF-121 adult hermaphrodite gonad arms. Marked are pachytene nuclei (arrowhead) and oocytes at diakinesis (short arrow). The first oocyte is outlined. (F) Abnormal sperm in the spermatheca of hsp-16p::RNF-121 adult hermaphrodite (arrow). (G and H) DTCs migration defects in hsp-16p::RNF-121 adults. Bar, 10 μm. (I) Percentage of animals with abnormal gonads. Analysis was performed to the transgenic lines (3 independent lines from each construct) hsp-16p::RNF-121, hsp-16p::RNF-121C222A223A, and hsp-16p::RNF-121 treated with der-1(RNAi). RNAi of der-1 in wild-type worms (N2 background) was performed as a control. Additional controls are N2 worms and transgenic worms expressing the E3 ligase RNF-5. Worms were treated with heat shock at mid-L2 stage to induce transgene expression (+) or nontreated controls (−). n = total number of worms analyzed in three to five independent experiments (indicated above each bar).
PAT-3::GFP; hsp-16p::RNF-121 double transgene after induction of RNF-121 expression during the first phase of gonad migration (mid-L2 stage). PAT-3::GFP levels are high in the ER of the gonad DTCs at this stage of development, whereas fluorescence intensity decreased after induction of RNF-121. The mean ratio of fluorescence intensity DTC/muscle (DTC values were normalized to the fluorescence intensity in the muscle perinuclear region; see Materials and Methods) was lower in the PAT-3::GFP; hsp-16p::RNF-121 worms than in the PAT-3::GFP control strain (0.39 ± 0.07 vs. 0.58 ± 0.11, respectively; n = 50 for each group; p < 0.0001) (Figure 5, A and B). This suggests that RNF-121 is able to ubiquitinate the ER-localized PAT-3::GFP in the DTCs and send it for proteasomal degradation in the cytoplasm. To test whether PAT-3::GFP is ubiquitinated by RNF-121, we performed in vitro ubiquitination assays. To this end, PAT-3::GFP was immunoprecipitated using anti-GFP antibody from rnf-121(ok848); PAT-3::HA::GFP mutant worms that contain a...
deletion in the rnf-121 coding sequence. The immune-purified PAT-3::GFP was then subjected to in vitro ubiquitination assay using bacterially expressed and purified GST-RNF-121RING in the presence of HA-tagged ubiquitin. This reaction resulted in the formation of high-molecular-weight conjugates that were recognized by antibodies to HA (recognizes both PAT-3 and HA-Ub) as well as antibodies to GFP (Figure 5C). Immune-purified PAT-3::GFP from PAT-3::GFP strain that does not include the rnf-121 deletion also could be ubiquitinated by wild-type RNF-121 but not by a RING finger mutant (Supplemental Figure S5). These data provide direct evidence for the ubiquitination of PAT-3::GFP by RNF-121.

To test whether PAT-3 is a main target of RNF-121 E3 ligase activity in the gonad, we analyzed young adults hsp-16::RNF-121;PAT-3::GFP double transgene after RNF-121 induction at the mid-L2 stage (Supplemental Figure S6). The percentage of worms with abnormal gonad was 2.8-fold lower in the double transgene (25.9% in hsp-16::RNF-121;PAT-3::GFP compared with 72.6% in hsp-16::RNF-121; Figure 4I and Supplemental Figure S6), indicating that high levels of PAT-3::GFP partially suppress the gonad defects caused by RNF-121 and suggesting that PAT-3 is a primary substrate of RNF-121 in the gonad.

We next tested PAT-3::GFP expression in rnf-121(RNAi)-treated worms and compared it to wild-type background. Few GFP inclusions (2.7 ± 0.9/worm) could be detected in 31.3 ± 9.0% of the PAT-3::GFP worms (n = 150) (Figure 5D, E and H); however, strong increase in fluorescence and accumulation of PAT-3::GFP inclusions (12.9 ± 2.9/worm) was detected in 70.7 ± 18.1% of the rnf-121(RNAi)-treated worms (n = 150) (Figure 5F, D and E). In accordance, massive accumulation of inclusions and high GFP levels were detected in PAT-3::GFP;rnf-121(ok848) mutant larvae (Figure 5F). The nature of inclusions and the dynamics of PAT-3::GFP molecules within the inclusions were analyzed by FRAP. Photobleach in a restricted area in the inclusion affected the entire structure implying that the molecules in the inclusion are mobile. However recovery of fluorescence was not detected after 7 min of analysis (as well as 40 min; data not shown) indicating that the misfolded PAT-3::GFP molecules within the inclusion do not exchange with a soluble pool of PAT-3::GFP (Figure 5G). Next, using a complementary FLIP analysis, we could not detect exchange with the cytosolic fraction, implying that the PAT-3::GFP molecules are tightly bound in the inclusions and suggesting that the inclusions are membrane bound (Figure 5H). The inclusion pattern differed from smaller inclusions that appeared after 2 μg/ml tunicamycin treatment, which probably reflected the retention of nonglycosylated PAT-3 precursors in the ER (n = 14) (Figure 5I).

The intense accumulation of PAT-3::GFP inclusions suggests that RNF-121 is essential for the regulated degradation of unassembled PAT-3::GFP subunits in the ER.

DISCUSSION

RNF-121 is a newly identified E3 ligase that is localized to the ER, is essential for larval development during ER stress conditions, and is capable to degrade a type I transmembrane ERAD substrate, PAT-3::GFP. Its predicted structure resembles the predicted structure of Hrd1/Synviolin (Kikkert et al., 2004), harboring six transmembrane domains that may be important for recognition of integral membrane substrates and a RING finger catalytic site exposed to the cytoplasmic ER surface. A difference between the predicted structures of the two proteins is that C terminus, which contains additional transmembrane domain in RNF-121. The variety of structurally similar ER-related E3 ligases in higher eukaryotes (Kostova et al., 2007) may suggest complementary roles at different stages of the ER stress response. For example, Hrd1 is thought to have a role in the nonadaptive ER stress response that lead cells to apoptosis, because it has been shown that Synovial cells in rheumatoid arthritis over-express Hrd1/Synviol and show resistance to ER-stress induced apoptosis (Amano et al., 2003). Alternatively, we demonstrate here that inactivation of RNF-121 is associated with increased ER stress at mild levels of tunicamycin, suggesting a role for RNF-121 in the adaptive response to ER stress. In contrast to Hrd1 and additional ERAD components that are regulated by the UPR on the transcriptional level through the IRE1-XBP1 pathway (Travers et al., 2000; Molinari et al., 2003; Yamamoto et al., 2008), the regulation of RNF-121 is through the PERK/PEK-1 pathway. We detected elevation in RNF-121 protein and not in its mRNA levels upon ER stress. Our data suggest that RNF-121 is regulated by PEK-1 by increased translation or protein stability probably through a tissue-specific downstream target of PEK-1. In the absence of PEK-1, RNF-121 protein levels are constantly high and are not further elevated after ER stress. It suggests that inactivation of PEK-1 causes constant ER stress that results in increased RNF-121 protein levels regulated through an alternative pathway, for example by ATF-6, which was shown to act redundantly to PEK-1 in C. elegans (Shen et al., 2005).

The germline defects that resulted in sterility after induction of RNF-121 at the mid-L2 stage suggest uncontrolled degradation of integrin subunits as well as additional regulators that mature in the ER and function in the developing somatic gonad. Substrates are expected to be expressed in somatic cells because RNF-121 expression is driven by an extrachromosomal array, which is subjected to germline suppression (A. Fire). Therefore, the observed effects are mainly a result of RNF-121 activity in the somatic cells of the gonad or in the body wall muscle cells that assemble the basal laminae on which the distal tip cells migrate. The somatic gonad at the L2 stage includes the two DTCs, the anchor cell, uterine precursor cells, and sheath/spermathecal precursor cells (SS cells) (McCarter et al., 1997). Laser ablation of the SS precursor cells has been shown to result in various germline abnormalities as reduced germline proliferation, defective exit from pachytene stage, feminization of the germline, and endomitotic oocytes (Emo) due to ovulation failure (McCarter et al., 1997). Induction of RNF-121 at this stage caused similar phenotypes as small gonad arms, delay in pachytene exit and abnormal sperm, suggesting damage to the SS precursor cells. Moreover, overexpression of RNF-121 caused severe abnormalities in gonad path that reflect defects in cell migration and therefore in the function of the DTCs. This suggests an association between RNF-121 and regulation of cell–extracellular matrix adhesion, probably by effecting the maturation and exit from the ER of factors required for controlled cell adhesion. Previous support for a possible link between the ERAD process and cell adhesion has been shown for the ubiquitin ligase gp78 that targets the transmembrane metastasis suppressor KAI1 for degradation (Tsai et al., 2007). We focused on PAT-3::GFP as the first identified substrate for RNF-121. Integrin-α and -β subunits are type I transmembrane glycoproteins that must be correctly assembled in the ER, transported to the Golgi, and finally transported to the plasma membrane as the mature assembled heterodimers. In mammalian cells, association of preintegrin-β1-chain with calnexin suggests that it is subjected to the ER protein quality control (Lenter and Vestwe-
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