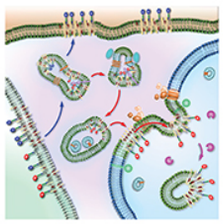
Autophagy



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REVIEW

How autophagy controls the intestinal epithelial barrier

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ABSTRACT

Macroautophagy/autophagy is a cellular catabolic process that results in lysosome-mediated recycling of organelles and protein aggregates, as well as the destruction of intracellular pathogens. Its role in the maintenance of the intestinal epithelium is of particular interest, as several autophagy-related genes have been associated with intestinal disease. Autophagy and its regulatory mechanisms are involved in both homeostasis and repair of the intestine, supporting intestinal barrier function in response to cellular stress through tight junction regulation and protection from cell death. Furthermore, a clear role has emerged for autophagy not only in secretory cells but also in intestinal stem cells, where it affects their metabolism, as well as their proliferative and regenerative capacity. Here, we review the physiological role of autophagy in the context of intestinal epithelial maintenance and how genetic mutations affecting autophagy contribute to the development of intestinal disease.

Abbreviations: AKT1S1: AKT1 substrate 1; AMBRA1: autophagy and beclin 1 regulator 1; AMPK: AMP-activated protein kinase; APC: APC regulator of WNT signaling pathway; ATF6: activating transcription factor 6; ATG: autophagy related; *atg16l1[ΔIEC]* mice: mice with a specific deletion of *Atg16l1* in intestinal epithelial cells; ATP: adenosine triphosphate; BECN1: beclin 1; bsk/Jnk: basket; CADPR: cyclic ADP ribose; CALCOCO2: calcium binding and coiled-coil domain 2; CASP3: caspase 3; CD: Crohn disease; CDH1/E-cadherin: cadherin 1; CF: cystic fibrosis; CFTR: CF transmembrane conductance regulator; CGAS: cyclic GMP-AMP synthase; CLDN2: claudin 2; CoPEC: colibactin-producing *E. coli*; CRC: colorectal cancer; CYP1A1: cytochrome P450 family 1 subfamily A member 1; DC: dendritic cell; DDIT3: DNA damage inducible transcript 3; DEPTOR: DEP domain containing MTOR interacting protein; DSS: dextran sulfate sodium; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; EIF2A: eukaryotic translation initiation factor 2A; EIF2AK3: eukaryotic translation initiation factor 2 alpha kinase 3; EIF2AK4/GCN2: eukaryotic translation initiation factor 2 alpha kinase 4; ER: endoplasmic reticulum; ERN1: endoplasmic reticulum to nucleus signaling 1; GABARAP: GABA type A receptor-associated protein; HMGB1: high mobility group box 1; HSPA5/GRP78: heat shock protein family A (Hsp70) member 5; IBD: inflammatory bowel disease; IEC: intestinal epithelial cell; IFN: interferon; IFNG/IFNγ:interferon gamma; IL: interleukin; IRGM: immunity related GTPase M; ISC: intestinal stem cell; LGR5: leucine rich repeat containing G protein-coupled receptor 5; LRRK2: leucine rich repeat kinase 2; MAP1LC3A/LC3: microtubule associated protein 1 light chain 3 alpha; MAPK/JNK: mitogen-activated protein kinase; MAPK14/p38 MAPK: mitogen-activated protein kinase 14; MAPKAP1: MAPK associated protein 1; MAVS: mitochondrial antiviral signaling protein; miRNA: microRNA; MLKL: mixed lineage kinase domain like pseudokinase; MLST8: MTOR associated protein, LST8 homolog; MNV: murine norovirus; MTOR: mechanistic target of rapamycin kinase; NBR1: NBR1 autophagy cargo receptor; NLRP: NLR family pyrin domain containing; NOD: nucleotide binding oligomerization domain containing; NRBF2: nuclear receptor binding factor 2; OPTN: optineurin; OXPHOS: oxidative phosphorylation; P: phosphorylation; Patj: PATJ crumbs cell polarity complex component; PE: phosphatidyl-ethanolamine; PI3K: phosphoinositide 3-kinase; PIK3C3/VPS34: phosphatidylinositol 3-kinase catalytic subunit type 3; PIK3R4: phosphoinositide-3-kinase regulatory subunit 4; PPARG: peroxisome proliferator activated receptor gamma; PRR5: proline rich 5; PRR5L: proline rich 5 like; PtdIns3K: phosphatidylinositol 3-kinase; PtdIns3P: phosphatidylinositol 3-phosphate; RB1CC1/FIP200: RB1 inducible coiled-coil 1; RER: rough endoplasmic reticulum; RHEB: Ras homolog, MTORC1 binding; RICTOR: RPTOR independent companion of MTOR complex 2; RIPK1: receptor interacting serine/threonine kinase 1; ROS: reactive oxygen species; RPTOR: regulatory associated protein of MTOR complex 1; RPS6KB1: ribosomal protein S6 kinase B1; SH3GLB1: SH3 domain containing GRB2 like, endophilin B1; SNP: single-nucleotide polymorphism; SQSTM1: sequestosome 1; STAT3: signal transducer and activator of transcription 3; STING1: stimulator of interferon response cGAMP interactor 1; TA: transit-amplifying; TFEB: transcription factor EB; TFE3: transcription factor binding to IGHM enhancer 3; TGM2: transglutaminase 2; TJ: tight junction; TJP1/ZO1: tight junction protein 1; TNBS: 2,4,6-trinitrobenzene sulfonic acid; TNF/TNFα: tumor necrosis factor; Tor: target of rapamycin; TRAF: TNF receptor associated factor; TRIM11: tripartite motif containing 11; TRP53:

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transformation related protein 53; TSC: TSC complex subunit; Ub: ubiquitin; UC: ulcerative colitis; ULK1: unc-51 like autophagy activating kinase 1; USO1/p115: USO1 vesicle transport factor; UVRAG: UV radiation resistance associated; WIPI: WD repeat domain, phosphoinositide interacting; WNT: WNT family member; XBP1: X-box binding protein 1; ZFYVE1/DFCP1: zinc finger FYVE–type containing 1.

Introduction

The intestine's first line of defense is conferred by a single layer of epithelial cells [1]. In the small intestine, the epithelium is structured into villi and the crypts of Lieberkühn, while the colon consists solely of crypts [1]. Along with their role in nutrient absorption and metabolism, intestinal epithelial cells (IEC) establish a mucosal barrier that protects the body from exogenous elements including pathogenic bacteria [1]. Having a general turnaround time of 4-5 d, IECs are continuously replenished by cycling LGR5^{+} intestinal stem cells (ISC) located at the base of the crypts [2]. In the small intestine, ISCs are intercalated by niche-supporting Paneth cells that maintain stemness and secrete antimicrobial factors [3]. Higher up in the crypt lie transit-amplifying (TA) cells, whose daughter cells develop into all differentiated IEC types: absorptive enterocytes, tuft cells, enteroendocrine cells, goblet cells, and Paneth cells [1]. A breakdown of this barrier is characteristic of intestinal pathologies, and in particular chronic inflammation of the gut known as inflammatory bowel disease (IBD), which is represented by Crohn disease (CD) and ulcerative colitis (UC) [4]. IBD is a multifactorial disease caused by a combination of genetic and environmental factors and, to date, more than 200 genetic risk loci have been identified [5].

As we will detail in this review, multiple lines of evidence demonstrate the critical link between intestinal function and autophagy. This relation is complex, as autophagy can dramatically regulate multiple aspects of intestinal physiology, from maintenance of the epithelial architecture to metabolic regulation, function of specific intestinal epithelial subsets, regulation of inflammatory pathways, and defense against infection (Table 1). The tight link between autophagy and intestinal physiology might be best illustrated by the observation of the strong association between genes of the autophagy pathway and susceptibility to CD; those genes include *ATG16L1* (Autophagy related 16 like 1), *IRGM* (immunity related GTPase M), *ULK1* (unc51–like like autophagy activating kinase 1), and *LRRK2* (leucine rich repeat kinase 2) [6–14]. Multiple review articles have provided an overview of the general role of autophagy in intestinal function [15-20]; here, we will focus specifically on the intestinal epithelial barrier, which serves not only as a first line of defense against enteric pathogens, but also plays essential roles in nutrient acquisition and in the crosstalk with the mucosal immune system. Indeed, while autophagy is certainly critical in regulating the function of most if not all cell types in the intestine, including innate and adaptive immune cells as well as stromal cells, a better understanding of the role of autophagy in IECs is rapidly emerging.

A brief outline of the autophagy process

The core autophagic machinery

The primary functions of autophagy (here specifically referring to macroautophagy) are to recycle and reuptake nutrients from the cytoplasm during conditions of metabolic stress [21] and to degrade specific cytosolic components, such as damaged mitochondria through mitophagy [22] or intracellular bacteria through xenophagy [23]. Autophagy involves the initial formation of a double-membrane structure, known as a phagophore, that engulfs the autophagy target and proceeds to form an enclosed autophagosome [24]. The autophagosome ultimately fuses with lysosomes to degrade its contents via enzymes and reactive oxygen species (ROS) and reactive nitrogen species (Figure 1) [24]. Autophagy is initiated by the ULK1 (unc–51 like autophagy activating kinase 1) complex (Atg1 in yeast), which phosphorylates downstream autophagic proteins [25-27]. Transmembrane-bound ATG9 (autophagy related 9) provides the double-membranes that form the phagophore and are largely derived from the endoplasmic reticulum (ER). Elongation of the phagophore membrane occurs through the production of phosphatidylinositol-3-phosphate (PtdIns3P) by the BECN1 (beclin 1)-phosphatidylinositol 3-kinase (PtdIns3K) complex followed by recruitment of the WIPI (WD repeat domain, phosphoinositide interacting) complex (Atg2-Atg18 in yeast) [28,29]. Two ubiquitin-like conjugation systems play key roles at later stages of the autophagic process. One of which, the ATG12 conjugation system, is comprised of the ATG12–ATG5-ATG16L1 complex and requires ATG7 and ATG10 activity for its formation [30]. This complex is necessary for autophagosome elongation [31] and recruitment of the second conjugation system comprising of the MAP1LC3A/LC3 (microtubule associated protein 1 light chain 3 alpha) and GABARAP family of proteins (GABA type A receptor-associated protein; Atg8 in yeast) [32,33]. MAP1LC3A or GABARAP is conjugated to phosphatidylethanolamine (PE) by ATG7 and ATG3, resulting in the maturation of the autophagosome [30]. Finally, autophagic targets can be specifically recruited to the autophagic machinery through cargo receptors, including CALCOCO2 (calcium binding and coiled-coil domain 2), SQSTM1 (sequestosome 1), NBR1 (NBR1 autophagy cargo receptor), and OPTN (optineurin) [24].

Molecular regulators of autophagy

The autophagy pathway requires regulation to best support cells during homeostasis, stress, and infection. Several factors converge to keep autophagic activity at bay while nutrients are sufficient. Most notably, autophagy is suppressed by MTOR (mechanistic target of rapamycin kinase) [34], a key metabolic

Table 1. Genetic models deciphering the role of autophagy in the intestinal epithelium.

Genetic Background (*species*)	Physiological Condition(s)/ Injury Model(s)	Altered Signaling Pathway(s)/ Major Intestinal Phenotype(s)	Reference(s)
ATG16L1^{T300A} (*Homo sapiens*)	CD patients	heightened ER stress in Paneth cells	Deuring et al., 2014 [86]
	CRC patients	better life expectancy, increased type I IFN	Grimm et al., 2016 [157]
		increased risk of CRC	Nicoli et al., 2014 [158]
XBP1 hypomorphic polymorphism (*Homo sapiens*)	CD patients	loss of Paneth cells, reduced antimicrobial peptide secretion	Kaser et al., 2008 [78]
atg14[ΔIEC] (*Mus musculus*)	Homeostasis	TNF-dependent spontaneous villus atrophy and intestinal enterocytes	Jung et al., 2019 [105]
atg16l1[ΔIEC] (*Mus musculus*)	Homeostasis	heightened ER stress,	Adolph et al., 2013 [69];
		spontaneous development of CD-like ileitis in aged mice,	Tschurtschenthaler et al., 2017 [65]
		defective Paneth cells	
	MNV infection followed by DSS-induced colitis	exaggerated intestinal cell death, TNF-mediated intestinal necroptosis, reduced Paneth cell numbers and other secretory cell-types	Matsuzawa-Ishimoto et al., 2017 [98]
	Helicobacter hepaticus infection coupled with IL10 blockade	excessive proinflammatory cytokine release, exacerbated tissue pathology, increased TNF-induced IEC apoptosis	Pott et al., 2018 [100]
	Citrobacter rodentium infection	no difference from wild type	Pott et al., 2018 [100]
		decreased infectious burden	Martin et al., 2018 [133]
	IL22 injection	TNF signaling-mediated CGAS-STING1 triggered RIPK3-MLKL axis dependent IEC necroptosis	Aden et al., 2019 [103]
	Radiation-induced injury	defective mitophagy, increased ROS in ISCs, impaired ISC regeneration, metabolic dysregulation	Levy et al., 2020 [77]
atg16l1[ΔIEC]; apc$^{Min/+}$	*apc*$^{Min/+}$ model of CRC with CoPEC infection	increased DNA damage, inflammation, and carcinogenesis	Lucas et al., 2020 [159]
atg16l1$^{fl/fl}$ *Defa4/ αDefensin4 IRES-cre* (*Mus musculus*)	Cigarette smoke model	enhanced TNF-mediated crypt base and Paneth cell apoptosis	Liu et al., 2018 [164]
atg16l1;xbp1[ΔIEC] (*Mus musculus*)	Homeostasis	increased NFKB and TNF signaling, exacerbated transmural inflammation	Adolph et al., 2013 [69]
	Homeostasis	develop early life transmural ileitis	Tschurtschenthaler et al., 2017 [65]
atg16l1^{HM} (*Mus musculus*)	Homeostasis	dysmorphic Paneth cells, decreased lysozyme secretion	Cadwell et al., 2008 [63]; Cadwell et al., 2009 [70]
		increased type I IFN	Marchiando et al., 2013 [132]; Martin et al., 2018 [133]
	MNV infection followed by DSS-induced acute colitis	TNF and IFNG/IFN-γ-mediated intestinal inflammation, proximal epithelial hyperplasia	Cadwell et al., 2010 [68]
	Citrobacter rodentium infection	decreased infectious burden	Marchiando et al., 2013 [132]; Martin et al., 2018 [133]
atg16l1^{T316A} (*Mus musculus*)	Homeostasis	defective Paneth cell and goblet cell morphology	Lassen et al., 2014 [74]
	Salmonella Typhimurium infection	decreased lysozyme secretion	Bel et al., 2017 [137]
	Citrobacter rodentium infection with Pac-1 treatment	decreased infectious burden	Martin et al., 2018 [133]
	Cigarette smoke model	metabolic dysregulation, enhanced crypt base apoptosis, defective Paneth cell morphology	Liu et al., 2018 [164]
atg4b$^{-/-}$ (*Mus musculus*)	DSS-induced acute colitis	increased proinflammatory cytokines, abnormal Paneth cells	Cabrera et al., 2013 [136]
	Citrobacter rodentium infection	decreased infectious burden	Martin et al., 2018 [133]
atg5[ΔIEC] (*Mus musculus*)	*Shigella* infection model	enhanced crypt cell death, increased epithelial shedding, ileal inflammation	Chang et al., 2013 [165]
	Salmonella Typhimurium infection	increased burden of infection	Benjamin et al., 2013 [66]
	Radiation-induced injury	defective mitophagy, increased ROS in ISCs, impaired ISCs regeneration	Asano et al., 2017 [76]
	Homeostasis	defective Paneth cells	Burger et al., 2018 [99]
	Toxoplasma gondii infection model	cytokine-mediated Paneth cell loss, TNF- and IFNG-driven intestinal cell death, reduced antimicrobial peptide expression	
atg5$^{fl/fl}$ *Defa4 IRES-cre* (*Mus musculus*)	*Toxoplasma gondii* infection model	Paneth cell loss	Burger et al., 2018 [99]
atg5$^{+/-}$*;apc*$^{Min/+}$ (*Mus musculus*)	*apc*$^{Min/+}$ model of CRC	increased tumor burden, better response to treatment with IFNG	Wang et al., 2015 [160]

(*Continued*)

Table 1. (Continued).

Genetic Background (*species*)	Physiological Condition(s)/ Injury Model(s)	Altered Signaling Pathway(s)/ Major Intestinal Phenotype(s)	Reference(s)
atg7[ΔIEC] (*Mus musculus*)	Homeostasis	defective Paneth cells	Wittkopf et al., 2012 [64]
	DSS-induced acute colitis	no difference from wild type	
	Citrobacter rodentium infection	no difference from wild type	
	Shigella infection model	increased pathology, increased infectious burden	Inoue et al., 2012 [166]
	Listeria monocytogenes infection	enhanced crypt cell death, increased epithelium shedding, ileal inflammation	Chang et al., 2013 [165]
		increased infectious burden	Sorbara et al., 2018 [58]
atg7$^{fl/fl}$ *Vil1-CreERT2* (*Mus musculus*)	Tamoxifen-induced IEC - specific deletion model	increased mitochondria defects, DNA damage, and ROS, more TRP53-mediated ISC apoptosis, defective crypt regeneration	Trentesaux et al., 2020 [106]
atg7[ΔIEC];apc$^{Min/+}$	*apc*$^{Min/+}$ model of CRC	decreased tumor burden, increased anti-tumor immune response	Lévy et al., 2015 [140]
atg7;xbp1[ΔIEC] (*Mus musculus*)	Homeostasis	increased NFKB/NFκB and TNF signaling, exacerbated transmural inflammation	Adolph et al., 2013 [69]
chuk$^{AA/AA}$ (*Mus musculus*)	DSS-induced acute colitis	heightened ER stress, less autophagy, defective Paneth cell morphology and goblet cell function	Diamanti et al., 2017 [167]
eif2ak4[ΔIEC] (*Mus musculus*)	Starvation-induced intestinal dysfunction	impaired autophagy, increased ROS, enhanced NLRP3 inflammasome-mediated IL1B production, increased Th17 response	Ravindran et al., 2016 [79]
hmgb1[ΔIEC] (*Mus musculus*)	DSS-induced acute colitis	increased IEC apoptosis	Zhu et al., 2015 [101]
irgm1$^{-/-}$ (*Mus musculus*)	Homeostasis	impaired autophagy and mitophagy, abnormal Paneth cells, reduced antimicrobial peptides	Liu et al., 2013 [71]
	DSS-induced acute colitis	increased focal epithelial denudation, mild goblet cell depletion,	Liu et al., 2013 [71]
		defective Paneth cell function, mild crypt epithelial cell hyperplasia	Rogala et al., 2018 [72]
lrrk2$^{-/-}$ (*Mus musculus*)	Homeostasis	defective lysosomal sorting, impaired Paneth cell autophagy	Zhang et al., 2015 [73]
map1lc3b$^{-/-}$	*Citrobacter rodentium* infection	decreased infectious burden	Martin et al., 2018 [133]
mtor[ΔIEC] (*Mus musculus*)	Homeostasis	no MTOR signaling, increased enteroendocrine cells, defective Paneth cells and goblet cells, villus blunting	Sampson et al., 2016 [118]
	Radiation-induced injury	impaired regeneration due to decreased ISC proliferation	
nlrp6$^{-/-}$ (*Mus musculus*)	Homeostasis	impaired autophagy, defective goblet cell function and mucus secretion	Wlodarska et al., 2014 [92]
rb1cc1/fip200[ΔIEC] (*Mus musculus*)	Homeostasis	TNF-dependent spontaneous villus atrophy and intestinal enterocyte loss	Jung et al., 2019 [105]
rictor[ΔIEC] (*Mus musculus*)	Homeostasis	no MTORC2 signaling, larger goblet cell size	Sampson et al., 2016 [118]
rptor[ΔIEC] (*Mus musculus*)	Homeostasis	no MTORC1 signaling, moderate ileal villus blunting, loss of Paneth cells increased enteroendocrine numbers	Sampson et al., 2016 [118]; Barron et al., 2017 [168]
rptor;rictor[ΔIEC] (*Mus musculus*)	Homeostasis	no MTOR signaling, severe villus flattening, decreased goblet cell size	Sampson et al., 2016 [118]
tfeb[ΔIEC] (*Mus musculus*)	Homeostasis	defective Paneth cells	Murano et al., 2017 [120]
	DSS-induced acute colitis	worsened pathology, barrier permeability	
tsc1[ΔIEC] (*Mus musculus*)	Small bowel resection (SBR)	enhanced MTOR signaling, robust villus growth, stronger crypt cell proliferation	Barron et al., 2017 [168]
	Homeostasis	enhanced MTOR signaling, increased crypt numbers and size	He et al., 2020 [128]
tsc1$^{fl/fl}$ *Lgr5-CreERT* (*Mus musculus*)	Tamoxifen-induced ISC-specific deletion model	enhanced MTOR-mediated MAPK14-TRP53 signaling, ISC/TA hyperproliferation	He et al., 2020 [128]

(*Continued*)

Table 1. (Continued).

Genetic Background (*species*)	Physiological Condition(s)/ Injury Model(s)	Altered Signaling Pathway(s)/ Major Intestinal Phenotype(s)	Reference(s)
xbp1[ΔIEC] (*Mus musculus*)	Homeostasis	increased autophagy, loss of Paneth cell and goblet cells, reduced antimicrobial peptide secretion, susceptible to *Listeria monocytogenes* infection, epithelial hyperproliferation, increased ISC numbers, enhanced TA proliferation	Kaser et al., 2008 [78]; Niederreiter et al., 2013 [83]; Adolph et al., 2013 [69]; Tschurtschenthaler et al., 2017 [65]
	DSS-induced acute colitis	increased TNF signaling, intestinal edema, erosion and crypt loss	Kaser et al., 2008 [78]
hmgb1[ΔIEC] (*Rattus norvegicus*)	Acute necrotizing pancreatitis model	excessive autophagy, increased degradation of TJP1/ZO-1, CLDN2 and OCLN in the intestine	Huang et al., 2019 [169]
Atg1 deletion mutant (*Drosophila melanogaster*)	Homeostasis	increased Tor activity, aberrant enlargement of IECs	Wen et al., 2017 [111]
Atg2, Atg3, Atg18 RNAi esg-specific expression (*Drosophila melanogaster*)	Homeostasis and upon DSS treatment	defective mitosis, decreased ISC numbers in midgut	Nagy et al., 2018 [123]
Atg6 ISC- and enteroblast-specific deletion mutant (*Drosophila melanogaster*)	Homeostasis	centrosome amplification and DNA damage accumulation, ISC hyperproliferation, intestinal hyperplasia	Na et al., 2018 [122]
Atg8a deletion mutant (*Drosophila melanogaster*)	Homeostasis	altered morphology ISCs, decreased ISC proliferation and regeneration	Nagy et al., 2018 [123]
Atg9 deletion mutant (*Drosophila melanogaster*)	Homeostasis	increased ROS activity, inhibition of bsk/Jnk signaling, impaired ISC proliferation	Tang et al., 2013 [87]
		increased Tor activity, aberrant enlargement of IECs	Wen et al., 2017 [111]
Atg13 deletion mutant (*Drosophila melanogaster*)	Homeostasis	increased Tor activity, aberrant enlargement of IECs	Wen et al., 2017 [111]
Atg14 RNAi esg-specific expression (*Drosophila melanogaster*)	Homeostasis and upon DSS treatment	defective mitosis, decreased ISC numbers in midgut	Nagy et al., 2018 [123]
Atg17/Fip200 deletion mutant (*Drosophila melanogaster*)	Homeostasis	increased Tor activity, aberrant enlargement of IECs	Wen et al., 2017 [111]
$Atg101^{6h}$ loss-of-function mutant (*Drosophila melanogaster*)	Homeostasis	shorter and thicker midguts, abnormal morphology with enlarged enterocytes, ISC hyperproliferation	Guo et al., 2019 [124]

regulator [35], and activated by the energy sensor, AMP-activated protein kinase (AMPK) (Figure 1) [36,37]. Autophagic recycling of cytosolic constituents releases lipids, sugars, and amino acids for cellular anabolism and energy production [38], linking it directly to cellular metabolism. Here we will briefly review the role of key autophagic regulators that we will later discuss in the context of IEC health.

MTOR

MTOR is a protein kinase that regulates cell growth and survival [35]. MTOR functions in two complexes. MTORC1 consists of MTOR, RPTOR (regulatory associated protein of MTOR complex 1) [35,39,40], MLST8 (MTOR associated protein, LST8 homolog), DEPTOR (DEP domain containing MTOR interacting protein), and AKT1S1 (AKT1 substrate 1) [35]. MTORC2 consists of MTOR, RICTOR (RPTOR independent companion of MTOR complex 2) [41], MLST8, DEPTOR, PRR5 (proline rich 5), PRR5L (proline rich 5 like), and MAPKAP1 (MAPK associated protein 1) [35]. Signaling through MTORC2 promotes cell proliferation and survival [35]. In contrast, MTORC1 positively regulates the synthesis of proteins, lipids, and nucleotides [35], and is a critical regulator of autophagy initiation [34]. MTORC1 inhibits the initiation of autophagy through direct

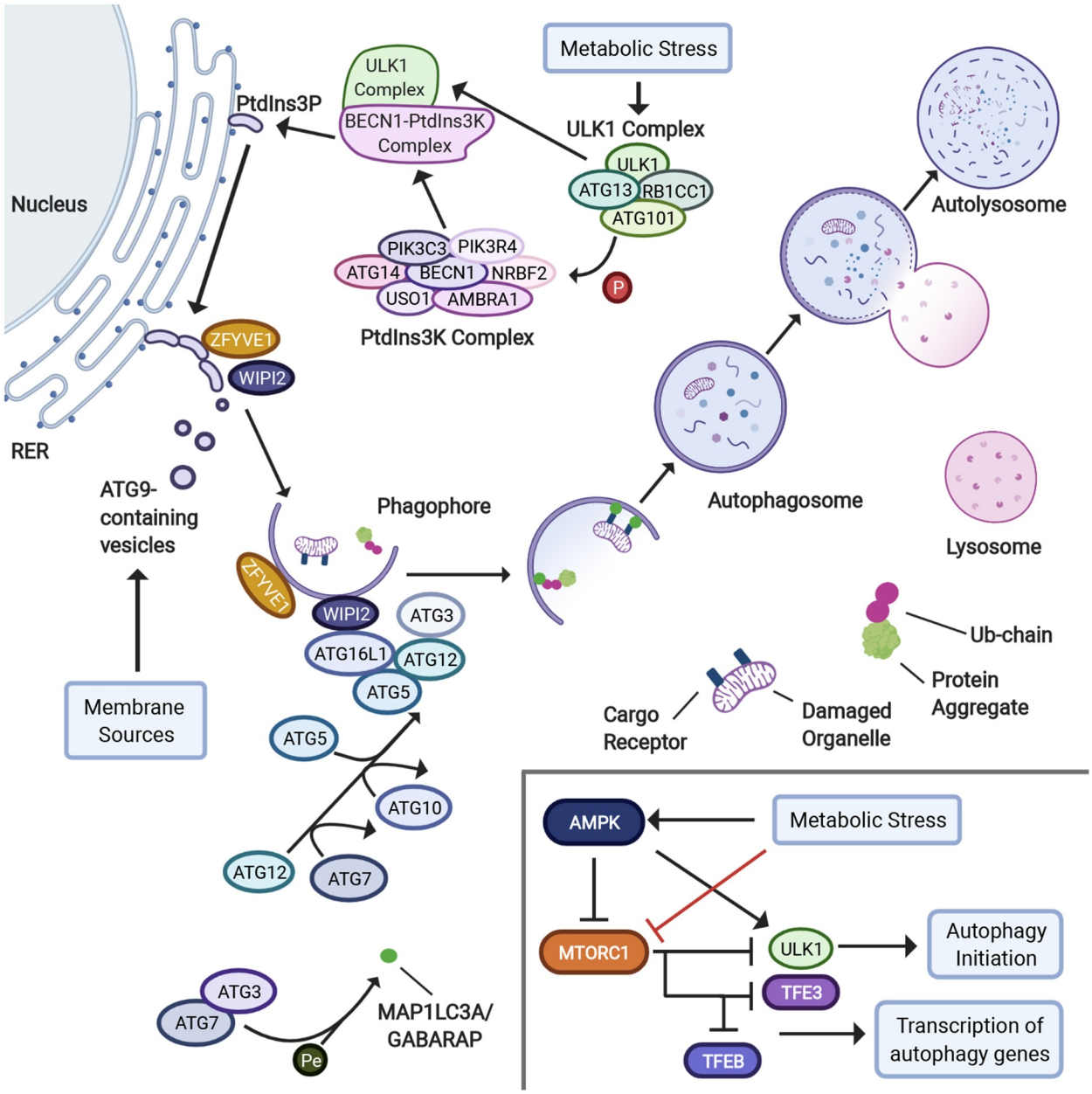

Figure 1. Overview of autophagy and autophagic regulation. In mammals, autophagy is initiated by the ULK1 complex [27], which, along with the PtdIns3K complex [28,29] participates in assembling the autophagic machinery on the rough ER. Additional complexes, such as the ATG12 conjugation system, assemble on the forming phagophore to which autophagic targets are recruited [31]. Targets can be labeled by ubiquitin chains, as well as cargo receptors, including SQSTM1, and CALCOCO2 [24]. After lysosomal fusion, the contents of the autolysosome are degraded, and nutrients are recycled by the cell [24]. The initiation of autophagy is tightly regulated by multiple members of the MTOR pathway, including AMPK [42], MTORC1 [42,43], TFEB and TFE3 [46-48]. AMBRA1: autophagy and beclin 1 regulator 1; NRBF2: nuclear receptor binding factor 2; P: phosphorylation; PE: phosphatidylethanolamine; PIK3C3/VPS34: phosphatidylinositol 3-kinase catalytic subunit type 3; PIK3R4: phosphoinositide-3-kinase regulatory subunit 4; RER: rough endoplasmic reticulum; Ub: ubiquitin; USO1/p115: USO1 vesicle transport factor; ZFYVE1/DFCP1: zinc finger FYVE-type containing 1.

phosphorylation of the ULK1 complex during nutrient abundance [42,43]. Conversely, in nutrient–deficient conditions, MTORC1's activity is downregulated and the complex is unable to inhibit autophagy initiation [44]. MTORC1 also regulates autophagy by inhibiting TFEB (transcription factor EB) [45], a transcriptional regulator of autophagy and lysosomal genes (further discussed below) [46-48]. Upstream regulation of MTORC1 is dependent on growth factor signaling, which suppresses the negative regulator activity of TSC1 (TSC complex subunit 1) and TSC2 [35]. TSC1 and TSC2 act by inhibiting RHEB (Ras homolog, mTORC1 binding) GTPase function [49,50], which is a direct activator of MTORC1 [49,51].

AMPK

AMPK is an upstream member of the MTOR pathway [37]. AMPK activation depends on the cellular energy state, where both a high ratio of adenosine monophosphate (AMP) to adenosine triphosphate (ATP) and low levels of glucose can activate AMPK [37]. Unlike MTORC1, AMPK positively regulates the ULK1 complex through phosphorylation when nutrients are restricted

[36]. Additionally, AMPK inhibits MTORC1 during conditions of nutrient stress [42]. Thus, AMPK positively regulates the initiation of autophagy both by activating the ULK1 complex and by inhibiting MTORC1 [52]. This is a cell–protective mechanism that increases the availability of nutrients through autophagy's catabolic activity [37].

TFEB

In 2009, TFEB was identified as a master regulator of lysosomal biogenesis genes and was quickly found to also play a major role in the transcriptional regulation of autophagy [46-48]. Along with the closely related protein, TFE3 (transcription factor binding to IGHM enhancer 3), TFEB positively regulates lysosomal biogenesis and autophagy by interacting with genes in the coordinated lysosomal expression and regulation gene network [53,54]. TFEB and TFE3 activity is tightly regulated by several kinases, most notably MTOR [45]. MTOR is a negative regulator of TFEB activity, thus when MTOR is active, components of the autophagic machinery are transcriptionally repressed.

Other regulators of autophagy

In addition to the above regulators, there are many other ways in which the cell monitors and controls autophagic flux. PtdIns3P is a product of class III PtdIns3K, and, as aforementioned, is essential for autophagy. However, the class I phosphoinositide 3-kinase (PI3K) products, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, inhibit autophagy through the PI3K-AKT-MTORC1 signaling axis, which maintains MTORC1 in an active state [55]. An additional method by which the cell regulates autophagy is through microRNAs (miRNAs) [56]. miRNAs are endogenously expressed short non-coding RNAs that post-transcriptionally regulate gene expression [57]. miRNAs have been shown to regulate a wide variety of steps in the autophagic process including inhibition of ULK1, MAP1LC3A, and ATG9 [56]. For more specialized forms of autophagy, including xenophagy and mitophagy, there are unique mechanisms that regulate steps in the autophagic process. For example, complement C3 as well as NOD1 (nucleotide binding oligomerization domain containing 1) and NOD2 can initiate autophagy through direct interaction with ATG16L1 upon detection of bacterial infection [58,59].

Autophagy and intestinal epithelial cell maintenance

Genetic and biochemical studies in experimental mouse models and cell culture, as well as clinical studies, have elucidated the involvement of autophagy in the maintenance of the intestinal epithelial barrier (Table 1). Tissue-specific knockouts of different autophagy proteins are used regularly, as they give insight into cell-intrinsic mechanisms, but also because global deletion of various autophagy proteins, such as ATG16L1 [60] and ATG5 [61], is lethal in mice [62]. Using these mouse models, it was shown, perhaps surprisingly given its importance in cell homeostasis, that autophagy deficiency within IECs does not result in spontaneous intestinal pathology [63,64], except for one report in aged mice with *Atg16l1* deletion in IECs [65] As we will discuss later, however, IEC deficiency in autophagy genes does lead to compromised protection against enteric infections [58,66,67]. Most strikingly, however, autophagy deficiency within the IEC compartment leads to an aberrant morphology of Paneth cells [63,68,69]. Indeed, IEC deficiency in *Atg5* [70], *Atg7* [64,70] and *Atg16l1* [63,68,69] as well as *Irgm1* [71,72] and *Lrrk2* [73], which also have been implicated in the autophagy pathway, all exhibit malformed and displaced granules within Paneth cells, suggesting dysfunctional secretion of the contents of these granules. Remarkably, humans and mice expressing the CD variant of *ATG16L1* also show abnormal Paneth cell morphology [74]. These dysfunctional Paneth cells not only compromise the secretion of barrier-protective antimicrobial peptides (as will be discussed later in the review) but are also impaired in their ability to support their neighboring ISCs [74]. ISCs rely on signaling by stem cell niche factors, such as WNT (WNT family member) and EGF (epidermal growth factor), to maintain stemness; these factors are in part, but not exclusively provided by Paneth cells [75]. In addition, ISC proliferation is not only essential for continuous IEC replenishment, but also vital in response to intestinal damage. Autophagy-deficient ISCs are more susceptible to excessive ROS [76,77] and lack the proliferative response necessary for IEC regeneration [76]. As we will detail below, the inability to cope with cell stress likely underscores the importance of autophagy in the maintenance of the epithelium and protection and recovery from infection and inflammation.

The interplay between autophagy and cellular stress in IECs

IECs encounter protracted biological and physicochemical stress, which leads to inflammation, cell damage, and loss of tissue function. In the past decade, several findings related to IBD have provided evidence that multiple cellular stress pathways, including the unfolded protein response (UPR) [78], integrated stress response (ISR) [79], and mechanical stress signaling [80], are tightly intertwined with autophagy in the maintenance of intestinal homeostasis during stress [81].

UPR

The accumulation of misfolded proteins in the ER activates the three branches of the UPR [81] through the protein chaperone, HSPA5/GRP78 (heat shock protein family A [Hsp70] member 5) [82]. These three branches are formed by ERN1/IRE1 (endoplasmic reticulum to nucleus signaling 1), EIF2AK3/PERK (eukaryotic translation initiation factor 2 alpha kinase 3), and ATF6 (activating transcription factor 6) [81]. A functional UPR response is crucial, as sustained ER stress disrupts intestinal homeostasis and alters the function of individual IEC subtypes. Lack of XBP1 (X-box binding protein 1), the master regulator of the UPR, leads to unresolved ER stress-mediated spontaneous enteritis in mice [78], characterized by a loss of Paneth cells, reduced goblet cells, and increased susceptibility to experimental colitis [65,69,78]. IEC-specific (ΔIEC) *xbp1* knockout mice (*xbp1[ΔIEC]*) mice also display enhanced ER stress associated with increased proliferation of ISCs and TA cells and increased susceptibility

to colorectal cancer (CRC) development [83]. ER stress can also be specifically induced in ISCs and TA cells through deletion of *Hspa5* by β-naphthoflavone induction of Cre recombinase under the *Cyp1a1* (cytochrome P450 family 1 subfamily A member 1, also known as Ah1Cre) promoter [84]. This leads to a loss of ISCs, promoting their differentiation into TA cells [84].

Autophagy was subsequently identified as a key protective mechanism during ER stress. *xbp1[ΔIEC]* mice also lacking autophagy (*atg7;xbp1[ΔIEC]* and *atg16l1;xbp1[ΔIEC]* composite knockout mice) experience exacerbated transmural inflammation due to hyperactivation of NFKB/NFκB through ERN1 and TNF (tumor necrosis factor) signaling [69]. Paneth cells are thought of as the drivers of pathology here, and both mice with elevated ER stress or autophagy deficiency exhibit an aberrant Paneth cell morphology [69,78]. Together, these studies infer that autophagy acts as a compensatory mechanism in IECs upon sustained ER stress.

The interplay of autophagy and ER stress is largely considered as a potential mechanism for the epithelial barrier breakdown in IBD. Cells from patients harboring a CD-associated genetic polymorphism in the *ATG16L1* gene (*ATG16L1*^{T300A}), which is known to render the protein less stable since it is more accessible to caspase cleavage [74,85], exhibit increased ER stress markers [86].

In mouse models, the absence of core autophagy proteins (ATG16L1 and ATG7) has also been linked to enhanced expression of ER stress markers like HSPA5, DDIT3 (DNA damage inducible transcript 3), and phospho-EIF2A (eukaryotic translation initiation factor 2A) [65,69,86]. While one group showed that *atg16l1[ΔIEC]* mice develop spontaneous CD-like ileitis in an age-dependent manner due to ERN1 accumulation [65], other reported models of colitis in autophagy-deficient animals require an additional mutation to affect the UPR pathway, as discussed above.

ROS and oxidative stress

IECs, especially ISCs, require autophagy to manage ROS-induced oxidative stress [76,77,87]. In *Drosophila*, Atg9

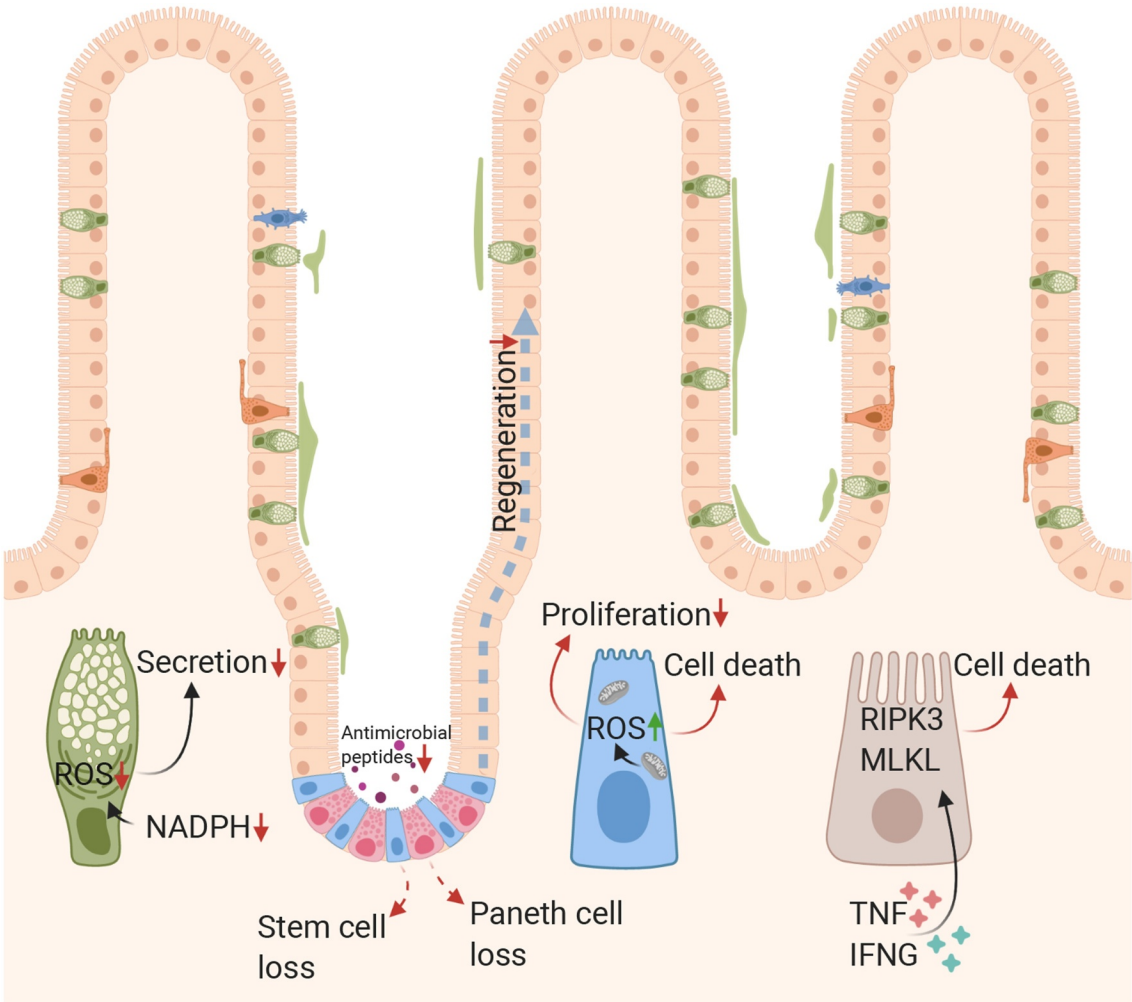

Figure 2. Intestinal epithelial cell-specific effects due to autophagy deficiency and intestinal injury. The autophagy–deficient intestinal epithelium is more susceptible to injury [76,98-100]. IECs require autophagy to protect from TNF- and IFNG–induced cell death [98-100], which is proposed to occur through RIPK3-MLKL-mediated necroptosis [98]. It has also been shown to specifically affect Paneth cells resulting in decreased antimicrobial peptides [99]. Autophagy-deficiency in ISCs results in increased ROS from mitochondrial defects, which leads to increased cell death [77] and reduced regeneration in response to irradiation [76]. Autophagy in goblet cells is required for ROS-mediated mucin secretion at baseline [91].

interacts with Traf6/Traf2 (TNF-receptor-associated factor 6) to activate bsk/Jnk (basket) signaling in response to oxidative stress and promote IEC renewal [87]. At the same time, ROS-induced autophagy acts as a negative feedback loop to regulate bsk signaling by dissociating Atg9 and Traf6 [87]. These findings were corroborated in mammalian cells as well [87]. Additionally, autophagy supports mitochondrial health and protects ISCs from irradiation-induced oxidative stress (Figure 2) [76,77]. Accordingly, *Atg5* deficiency in mice leads to an ISC-intrinsic impairment in intestinal regeneration following irradiation due to ROS buildup fueled by accumulated defective mitochondria; this effect is abrogated by antioxidant treatment [76]. More recent work has highlighted a link between bacterial sensing and autophagy in stem cell survival during intestinal damage; muramyl dipeptide sensing by NOD2 promotes ATG16L1-dependent mitophagy to protect ISCs from irradiation-induced ROS [77].

The role of mitophagy in ISCs is of additional interest, as ISCs rely on oxidative phosphorylation (OXPHOS) as their primary source of ATP [88]. In turn, mitochondrial ROS generated during OXPHOS promotes the differentiation of their daughter cells through MAPK14/p38 MAPK (mitogen-activated protein kinase 14) phosphorylation [88]. The reliance of ISC on mitochondrial OXPHOS for energy production suggests that mitochondria are crucial for ISC function. Indeed, mitochondrial health has been tied to ISC homeostatic function [89,90], and deficiency in the mitochondrial UPR leads to the loss of affected ISCs [90]. As autophagy functions through mitophagy to clear cells of defective mitochondria [22], it is of interest to further explore how autophagy deficiency may directly affect ISC metabolism.

In addition, studies have shown that cellular stress resulting from ROS has a goblet cell-specific function in mucin secretion that depends on autophagy. Mice lacking *Atg5* are impaired in mucin secretion, which leads to morphologically larger goblet cells than seen in their wildtype counterparts (Figure 2); however, goblet cell numbers stay unaffected, indicating that their differentiation at homeostasis is not influenced [91]. Addition of exogenous ROS improves mucin secretion in the autophagy–deficient goblet cells [91]. Furthermore, autophagy has been proposed to act downstream of NLRP6 (NLR family pyrin domain containing 6) activation in goblet cell mucin secretion [92], yet this remains to be proven in a microbially controlled setting with the use of littermate mice.

Response to metabolic stress

Modulation of nutrient availability and consequent impact on MTOR activity can affect ISC proliferation [93-95]. Indeed, fasting in *Drosophila* reduces overall IEC numbers through insulin-dependent signaling in ISCs, a phenomenon that is reversed through feeding [93]. In contrast, calorie restriction in mice leads to an increase in ISCs and Paneth cells [94,95]. Accordingly, inhibition of MTORC1 was proposed to increase cyclic ADP ribose (cADPR) production by Paneth cells, which drives ISC proliferation [95]. Later, work by Igarashi and Guarente [94] provided deeper insight by showing that MTORC1 inhibition in Paneth cells leads to a cascade of effects in ISCs resulting in increased RPS6KB1 (ribosomal protein S6 kinase B1) phosphorylation, which is consistent with sustained MTORC1 activity in ISCs [94]. Whether autophagy per se was impacted in this model, however, is unknown since these studies did not address whether fasting-induced autophagy can also affect Paneth cell and ISC functions. Given that ISCs are influenced both by mitochondrial health and nutrient availability, it is therefore likely that autophagy in ISCs affects their metabolic state.

Other cellular stresses

Autophagy has also been implicated in additional stress responses [80,96]. In an acute and spontaneous model of colitis, hypoxia was shown to ameliorate intestinal inflammation by downregulating the binding of MTOR and NLRP3 and activating autophagy [96]. In addition, actin-rich microvillar protrusions trigger a shear stress response that induces noncanonical autophagy in intestinal epithelial monolayers [80]. This facilitates the macroscopic transport of fluids across IECs and modulates gut permeability [80]. Acute amino acid starvation-induced autophagy dampens intestinal inflammation by a mechanism dependent on EIF2AK4/GCN2 (eukaryotic translation initiation factor 2 alpha kinase 4), a key mediator of the ISR [79]. Likewise, impaired autophagy in *Eif2ak4*-deficient IECs during starvation is associated with enhanced inflammation with increased ROS, NLRP3 inflammasome-mediated IL1B production, and a T helper 17 (Th17) cell response [79].

Collectively, these studies support the idea that crosstalk between cellular stress pathways and autophagy can promote intestinal homeostasis. Autophagy can protect cells and promote survival during conditions of stress, for example, by clearing misfolded protein aggregates and preventing apoptosis due to ER stress [81,97].

Autophagy mitigates intestinal cell death and promotes gut barrier function

As discussed above, regulation of cellular stress by autophagy and protection from stress–induced apoptosis is one aspect of how autophagy plays a role in IEC death [81,97]. Autophagy and autophagic proteins have also been shown to regulate epithelial apoptosis and necroptosis during intestinal injury and inflammation [98-101]. Protection from cell death is crucial for maintaining a functional epithelial barrier that protects the body from the intestine's contents, including the microbiota [1].

Recent studies from three independent groups have highlighted a role for autophagy in protecting the intestinal epithelium from cytokine–mediated cell death, especially in the context of intestinal infection [98-100]. Specifically, TNF and IFNG/IFNγ (interferon gamma), which are released during intestinal damage, were shown to drive both apoptosis and necroptosis in different colitis models (Figure 2) [98,100]. *Toxoplasma gondii* infection in *atg5[ΔIEC]* mice

demonstrated that Paneth cell-intrinsic autophagy prevents TNF- and IFNG-driven intestinal cell death in this model, but the type of cell death remains unclear [99]. In terms of bacterial triggers of inflammation, autophagy was shown to protect from apoptosis in a *Helicobacter hepaticus* infection model coupled with IL10 blockade [100]. Here, *atg16l1[ΔIEC]* mice were characterized by excessive inflammatory cytokine release, exacerbated tissue pathology, and increased TNF-induced IEC apoptosis [100]. Finally, autophagy was found to protect from necroptosis associated with chemical-induced colitis in murine norovirus (MNV)-infected mice [98]. Specifically, *atg16l1[ΔIEC]* mice have increased TNF-mediated epithelial necroptosis through RIPK1 (receptor interacting serine/threonine kinase 1), RIPK3, and MLKL (mixed lineage kinase domain like pseudokinase) signaling [98]. Additional insights into the mechanism showed that selective autophagic degradation of RIPK3 suppresses necroptosis [102]. RIPK3 is targeted for degradation through ubiquitination mediated by TRIM11 (tripartite motif containing 11) [102]. In turn, augmented RIPK3 activity and autophagy inhibition lead to necroptosis in mice with hyperactive MTOR signaling, induced by either a western diet or knockout of *Tsc1* [102].

IL22, which is a pleiotropic cytokine with both pro-regenerative and pro-inflammatory properties, depending on the context, can drive intestinal inflammation in an autophagy-dependent manner [103]. IECs express the receptor for IL22 and IL22-induced signaling within these cells leads to DNA damage and detection of cytosolic dsDNA by CGAS (cyclic GMP-AMP synthase)-STING1 (stimulator of interferon response cGAMP interactor 1), which is amplified by the loss of ATG16L1 [103]. This in turn results in increased type I IFN (interferon) and TNF, which drive MLKL-dependent necroptosis [103].

Autophagy induction has also been shown to be protective during inflammation-induced cell death. Beyond their role in autophagy, both BECN1 and ATG5 are associated with the initiation of apoptosis [101]. HMGB1 (high mobility group box 1), a chromatin-associated nuclear protein that also serves as an extracellular damage/danger-associated molecular pattern, is a critical regulator of autophagy through its interaction with BECN1 [104]. HMGB1 prevents calpain-mediated BECN1 and ATG5 cleavage, thereby restricting the switch between the pro-autophagic to pro-apoptotic function of BECN1 and ATG5 during inflammation and limiting apoptosis [101]. This is protective during intestinal inflammation by preventing apoptosis, as shown in *hmgb1[ΔIEC]* mice [101]. Intestinal tissue from IBD patients with active colitis is also characterized by decreased intracellular HMGB1 and increased pro-apoptotic BECN1 and ATG5 function, resulting in increased IEC apoptosis [101].

Intriguingly, spontaneous disruption of intestinal barrier function through IEC apoptosis in the absence of a colitis trigger, but in a background of autophagy deficiency, has also been reported. *atg14[ΔIEC]* mice show spontaneous villus atrophy and TNF-dependent apoptosis in IECs [105]. Likewise, conditional deletion of *RB1CC1/FIP200* (RB1 inducible coiled-coil 1) shows a similar phenotype [105]. In addition, tamoxifen-induced *Atg7* deletion causes a TRP53 (transformation related protein 53)-dependent increase in crypt cell apoptosis, associated with mitochondria defects and DNA damage [106]. Fasting autophagy-sufficient mice and thus inducing autophagy protects ISCs from oxaliplatin- and doxorubicin-induced DNA damage and apoptosis [106]. Taken together, these genetic studies using distinct mouse models of intestinal damage have provided compelling evidence for the conserved cytoprotective function of autophagy in the intestine at homeostasis and in inflammatory conditions.

Regulation of intestinal tight junctions by autophagy

Intestinal permeability to essential ions, nutrients, and water, but resistance to bacterial toxins and pathogens is controlled by tight junction (TJ) proteins [107]. TJ modulation, resulting in increased barrier permeability, has been associated with IBD [108], and research has highlighted a role for autophagy in regulating TJ proteins and in barrier maintenance [109].

Induction of autophagy through nutrient starvation reduces paracellular permeability of the intestinal epithelium by targeting the cation-selective and pore-forming TJ protein CLDN2 (claudin 2) for lysosomal degradation, by a process dependent on ATG16L1 and ATG7 [109]. In return, TNF-mediated inhibition of autophagy increases CLDN2 expression, thereby contributing to epithelial TJ dysfunction and increased barrier permeability [110]. Similarly, autophagy deficiency in *Drosophila*, namely due to deficiencies in Atg9, Atg1, Atg13, and Atg17/FIP200, results in increased intestinal barrier permeability [111]. While Atg9 is known to interact with the TJ protein, Patj, to regulate Tor (target of rapamycin) signaling and cell growth, how this leads to increased intestinal permeability has not been explored [111].

In vitro cell line data have also shown the regulation of OCLN (occludin), a critical component of TJ complexes, through an autophagy-independent interaction between BECN1 and OCLN [112]. This interaction at the plasma membrane results in the endocytosis of OCLN, which increases permeability [112]. This is countered by the induction of autophagy, which terminates constitutive BECN1-mediated endocytosis of OCLN, thus enhancing surface expression of OCLN and maintaining intestinal barrier integrity [112].

Autophagy has also been implicated in modulating TJs to support IEC-dendritic cell (DC) interactions during luminal antigen sampling and to prevent changes in barrier permeability [113]. Intestinal DCs sample and process luminal antigens by protruding dendrites through the epithelium. Autophagy reduces OCLN and CDH1/E-cadherin (cadherin 1) levels to support successful DC antigen sampling [113]. Consequently, inhibition of autophagy in either IECs, DCs, or both leads to impaired IEC-DC interactions, reduced antigen sampling, and a more pro-inflammatory DC phenotype [113].

Altogether, these findings highlight how autophagy-mediated TJ regulation contributes to intestinal barrier function and implicate autophagy deficiency as a potential driver of paracellular permeability in intestinal disease.

Autophagy regulators in homeostasis and disease

The regulation of autophagy in IECs encompasses a complex engagement of multiple signaling pathways [114]. Inflammatory cues, nutrient starvation, and cellular stress are all involved in the activation of autophagy, primarily through the MTOR signaling pathway [114,115]. Intestinal disease models in mice with genetic alterations of autophagy and its regulators have helped elucidate their role in intestinal homeostasis. Indeed, MTOR is activated in the intestines of mice with acute colitis [116,117] and MTORC1-dependent STAT3 (signal transducer and activator of transcription 3) signaling alleviates pathology [116]. In the small intestine, MTORC1 supports enterocytes, goblet and Paneth cells, and its deficiency results in stunted villi and increased enteroendocrine cells. Stem cell proliferation, however, is only affected in injury; consequently, the regeneration of the epithelium after irradiation injury is compromised in this model [118]. MTOR modulation of autophagy has also been observed to have intestinal effects. Indeed, MTOR-dependent suppression of autophagy contributes to intestinal inflammation and oxidative stress injury in lipopolysaccharide-induced experimental colitis and in IECs of active UC patients [119]. Activation of autophagy in the lipopolysaccharide-induced experimental colitis model ameliorates intestinal pathology [119]. Furthermore, in mice, IEC deficiency of the transcriptional regulator of autophagy TFEB reveals that TFEB is required for the restoration of the intestinal barrier post epithelial injury and colitis but dispensable at steady–state [120].

MTOR signaling [111,121] and autophagy [121-126] are both implicated in ISC renewal and differentiation. In *Drosophila*, loss of Atg9 and other autophagy-related proteins leads to increased Tor signaling resulting in aberrantly enlarged IECs, but no change in cell numbers. Accordingly, Tor inhibition through rapamycin and the consequent induction of autophagy reverts this phenotype [111]. Age-related changes in ISCs, including a decrease in overall numbers and in function [127], are also affected by MTOR and autophagy. In *Drosophila*, aged ISCs have a disrupted protein homeostasis, in part due to the loss of a "proteostatic checkpoint," which can be alleviated by inducing autophagy [125]. Moreover, rapamycin treatment and induction of autophagy decrease ISC proliferation in aging *Drosophila* [121]. In line with these findings, induced deficiency in Atg6, the *Drosophila* ortholog of mammalian BECN1, results in a premature age-related phenotype characterized by DNA damage and hyper-proliferation [122]. In mice, on the other hand, aging results in a decreased number of crypts and TA cells that correlates with increased MTOR activation and inhibiting MTOR results in a partial rescue of crypt numbers and proliferative cells [128].

Pleiotropic effects of autophagy in intestinal infection

Autophagy's role in the control of intestinal bacterial pathogens remains controversial since studies have revealed both beneficial and detrimental effects of autophagy on the outcomes of infection. In terms of infection control, xenophagy acts to restrict cyto-invasive pathogens; consequently, autophagy deficiency within IECs results in increased susceptibility to infection [58,66,67,129,130]. For example, the CD-associated adherent-invasive *Escherichia coli* can persist in autophagy-deficient IECs [129,130] and mice with autophagy specifically deleted in IECs demonstrate that autophagy is required for clearance and control of the dissemination of *Salmonella enterica* Typhimurium [66,67]. Our group showed that complement C3 coating of adherent-invasive *Escherichia coli* as well as the cyto-invasive Gram-positive pathogen, *Listeria monocytogenes*, targets these bacteria for xenophagic degradation and both *C3*- and *Atg7*-deficient mice have a higher burden of intestinal infection with *L. monocytogenes* [58].

Contrary to a beneficial role of autophagy during infection, studies have also shown that autophagy can contribute to an increased bacterial burden, and this, importantly, appears to be in the context of extracellular bacterial infection. Using *Citrobacter rodentium*, which is an extracellular bacterial pathogen of mice that induces Th17-associated inflammation and crypt hyperplasia [131], autophagy deficiency in *Atg16l1* hypomorphic mice was shown to lower the bacterial burden and protect mice from severe inflammation compared to their wild-type counterparts [132,133]. Interestingly, these findings parallel studies in the bladder where *Atg16l1* deficiency was also shown to be protective against the extracellular pathogen, uropathogenic *E. coli* [134]. Mechanistically, autophagy deficiency in the *C. rodentium* model results in increased immune activation, specifically associated with NOD2 and type I IFN signaling [132,133]. This is in line with the finding that ATG16L1 suppresses pro-inflammatory cytokine signaling downstream of NOD1 and NOD2 [135].

As mentioned briefly above, autophagy affects the antimicrobial response of Paneth cells and in this way, may also impact bacterial infection. Mice hypomorphic for *Atg16l1* have an altered Paneth cell morphology with irregular dense–core vesicles, which are required for antimicrobial secretion, resulting in diminished lysozyme secretion into the lumen [63]. Further studies of deficiency in autophagy genes, including *Atg5* [70], *Atg7* [64,70], *Atg4b* [136], *Irgm1* [71,72], and *Atg16l1* [67,69], have similarly reported altered Paneth cell morphology [64,67,69-72,136]. It is becoming apparent that autophagy deficiency affects the formation of secretory granules and the secretion of lysozyme, yet it is not clear how these changes occur and what their impact is on antimicrobial defense. Interestingly, an additional mechanism for autophagy has been identified in the unconventional secretion of lysozyme [137]. This specialized form of lysozyme secretion dependent on the autophagic machinery was found in IECs of mice infected with *S.* Typhimurium [137]. *S.* Typhimurium infection leads to a block in conventional secretion that results in autophagy-dependent secretion of lysozyme [137], a process absent in autophagy-deficient Paneth cells [137]. Interestingly, other antimicrobials are not secreted in this manner [137]. *In vitro*, supernatant from crypts derived from autophagy-deficient mice was unable to kill *S.* Typhimurium, indicating a role for secretory autophagy in the antimicrobial response [137]. While the above studies suggest that autophagy-deficient Paneth cells are impaired in their secretion of lysozyme, it is not clear whether this has far–

reaching effects on the gut microbiota nor whether it directly exacerbates intestinal infections. *LRRK2*, where variants of this gene have been linked with Parkinson disease as well as CD [12-14], is associated with the induction of autophagy with specific effects in Paneth cells [125]; work in *lrrk2*$^{-/-}$ mice indicates a NOD2-dependent role for LRRK2 in the packaging of lysozyme in dense-core vesicles [126]. This finding implicates the microbiota as a regulator of Paneth cell antimicrobial secretory function due to NOD2's role as a microbial sensor [126]. However, it has been proposed that LRRK2 plays a greater role in the immune cell compartment, rather than the epithelium; therefore, whether LRRK2 is acting in a cell-intrinsic manner to promote Paneth cell secretory functions is yet to be elucidated [127].

Together, autophagy proteins appear to play a significant role in the host response to infection in the intestinal epithelium, both through xenophagy and through other immunomodulatory mechanisms, resulting in both protection and lack thereof during intestinal infection. However, these roles appear to be pathogen- and context-specific.

The role of autophagy during intestinal disease

Genome-wide association studies (GWAS) led to the discovery of single-nucleotide polymorphisms (SNPs) within genes associated with the autophagy pathway and highlighted autophagy's role in disease. While there are also increased risks for extra-intestinal diseases, e.g. systemic lupus erythematosus [138], autophagy has been implicated within the intestine in the pathology of Crohn disease (CD) [15,139] as well as colorectal cancer (CRC) [140,141]. Furthermore, we also discuss the role of autophagy within intestinal manifestations of cystic fibrosis (CF) [142,143].

Polymorphisms of autophagy genes confer susceptibility to Crohn disease

IBD is a chronic inflammatory disorder of the gastrointestinal tract; broadly, CD may affect any part of the intestinal tract with discontinuous penetrating lesions, whereas UC is limited to the colon and rectum with continuous ulceration of the mucosal surface [4]. Several perturbations contribute to its pathogenesis, including a defective mucosal barrier, dysregulation of the immune response, and an altered microbiota composition [4]. Genetic polymorphisms in *NOD2* were the first to be associated with risk of CD development and remain the highest genetic risk–conferring factors [144-146]. As briefly discussed, NOD2 and autophagy cooperate within the xenophagic response, and ATG16L1 dampens pro-inflammatory signaling downstream of NOD2 [135,147]. It is of interest that variants of autophagy proteins have also been found to confer risk of developing CD, highlighting the potential interplay between these two pathways in CD. The role of polymorphisms targeting specific autophagy genes and conferring susceptibility to CD has been extensively reviewed in the literature [15,139]. Here we will focus on *ATG16L1, IRGM*, and *LRRK2*.

Among the polymorphisms of *ATG16L1* linked to CD, a SNP encoding a missense variant (rs2241880) has been most highly associated with this disease [6,8,9]. This SNP causes a codon change in exon 8 and a resulting substitution of threonine to alanine at position 300 within the protein (T300A). The site of the T300A substitution constitutes a caspase cleavage motif in ATG16L1 [74,85]. Activation of CASP3 (caspase 3) in the presence of the common risk allele accelerates the degradation of ATG16L1, resulting in impaired autophagy and increased cellular stress [74,85]. This particular SNP is found to be prevalent among various populations with an average of 43.9% of individuals heterozygous and 17.7% homozygous for the risk allele [148]. Prevalence is highest among people of South Asian and European descent where at least 25% are homozygous [148]. Of interest, SNPs in *ATG16L1* have not been identified to confer IBD risk in studies of Asian populations [149]. Additionally, *ATG16L1*^{T300A} has not been associated with Paneth cell defects in Japanese CD patients; rather, a *LRRK2* polymorphism was associated with defects in these cells within this population [150].

IRGM polymorphisms (rs13361189, rs4958847, and rs11741861) are known to increase CD and UC susceptibility [7,10,151]. IRGM belongs to the p47 immunity-related GTPase family, and its mouse homolog, IRGM1, critically controls intracellular pathogens by xenophagy [152]. Accordingly, *Irgm1*-deficient mice show a markedly increased susceptibility to chemical–induced colitis, a model that is largely driven by the accessibility of the tissue to commensal bacteria [71,72].

While SNPs in *LRRK2* are best known for their association with the development of Parkinson disease, *LRRK2* variants also confer risk for the development of CD [12-14]. Evidence suggests that LRRK2 controls a non-canonical autophagy pathway, alternative and parallel to that regulated by MTOR and ULK1, but dependent on the presence of an active BECN1 complex [153]. Through this pathway, LRRK2 is thought to modulate aggregated protein complexes for autophagy clearance [153]. The G2019S mutation in LRRK2, initially identified for its association with Parkinson disease, results in hyperactive kinase activity of the protein [13]. Interestingly, this variant and a closely linked mutation, N2081D, also increase LRRK2 kinase activity and confer genetic risk for CD [13,14].

Finally, most of our understanding of IBD genetics is from studying largely white European and East Asian descent populations. These studies have uncovered distinct genetic risk loci and a stronger association of autophagy with IBD risk in the former [149,154]. Interestingly, two genome-wide scans of African-American CD and UC patients and healthy controls did not identify SNPs in autophagy genes with increased CD risk [155], indicating there is still a lot to be learned about autophagy in IBD risk in different populations.

Influence of autophagy in colorectal cancer

Colorectal cancer (CRC) originates in the colon from hyperproliferative epithelial cells as a result of accumulated genetic and epigenetic mutations; these IECs can form initially benign adenomas that can progress into malignant carcinomas [156]. Altered expression and/or mutations of autophagy genes have

been reported in human gastric cancer and CRC, including genetic variations of *BECN1, UVRAG* (UV radiation resistance associated), *SH3GLB1* (SH3 domain containing GRB2 like, endophilin B1), *ATG10*, and *ATG5* [141]. Autophagy activity was shown to be increased in human CRC [140,141], suggesting it plays a role in the development and/or progression of CRC. Additionally, IBD patients are at a higher risk of later developing CRC and, as discussed, SNPs in autophagy-related genes have been associated with the pathogenesis of CD [141].

Patient data has suggested that the CD-associated $ATG16L1^{T300A}$ variant is protective within cancer, although the mechanism is currently unknown [157]. CRC patients that express the $ATG16L1^{T300A}$ variant have a better life expectancy than patients who have the $ATG16L1^{T300T}$ genotype [157]. A potential mechanism is through higher type I IFN production through the MAVS (mitochondrial antiviral signaling protein) pathway in the $ATG16L1^{T300A}$ background, which may protect through immune activation, but this has not been further explored in this context [157]. Further research in this area is warranted, however, especially in light of the findings from an earlier study that showed an increased, rather than a decreased risk of CRC in patients with the $ATG16L1^{T300A}$ mutation [158].

Mouse models have aimed to address the role of autophagy in CRC. The $apc^{Min/+}$ model, a mouse model of human familial adenomatous polyposis, results in tumorigenesis due to overactive WNT signaling and IEC proliferation caused by the deficiency in APC (APC regulator of WNT signaling pathway) [140,159,160]. $apc^{Min/+}$ mice with a heterozygous deletion for *Atg5* have an increased tumor burden compared to $apc^{Min/+}$ mice expressing both copies of *Atg5* [160]. At the same time, IFNG treatment is more effective in $apc^{Min/+}$;$atg5^{+/-}$ mice than in $apc^{Min/+}$;$atg5^{+/+}$ mice [160]. IFNG inhibits EGFR (epidermal growth factor receptor) and WNT signaling, which suppresses tumor cell proliferation [160]. *Atg5* heterozygosity activates these two pathways, as such IFNG is more effective [160]. This finding may have direct therapeutic implications since nearly 23% of CRC patients have a loss of *ATG5* expression in tumor cells [160]. Autophagy is protective in $apc^{Min/+}$ mice simultaneously infected with colibactin-producing *E. coli* (CoPEC), which are pathogens prevalent in CRC tumors [159]. CoPEC induces autophagy, which protects from CoPEC-induced DNA damage, inflammation, and carcinogenesis [159]. This highlights a role for autophagy in protecting against the effects of bacterial drivers of tumorigenesis as CoPEC-infected $apc^{Min/+}$ mice with deficiency in *Atg16l1* have worse outcomes than autophagy-sufficient mice [159]. Interestingly, $apc^{Min/+}$ mice deficient in *Atg7* in IECs show decreased tumor counts and an increased microbiota-dependent anti-tumor immune response [140]. This implicates autophagy deficiency in the protection from initial tumor development and potentially progression [140]. It appears that autophagy can affect CRC development and progression at different stages and in different contexts. While the $apc^{Min/+}$;*atg7[ΔIEC]* mouse model indicates that IEC autophagy deficiency is protective in initial development and progression of CRC [140], in line with the limited human data available [157], autophagy deficiency may be detrimental in the context of pathogenic drivers of tumorigenesis [159]. In addition, the *Atg5* mouse model displayed an increase in tumor burden [160]; however, autophagy activity appeared unaffected in the tumors, indicating that a potential autophagy-independent mechanism of ATG5 or an extra-tumoral response is affecting tumorigenesis.

Finally, understanding the specific effects of autophagy in CRC is crucial for the development of treatments. Autophagy, PI3K, and MTOR are all considered targets of interest in CRC therapy development; this has been reviewed extensively elsewhere [141]. For example, an MTORC1 and MTORC2 dual inhibitor (AZD-2014) has been shown to significantly inhibit cancer cell growth through autophagic cell death both *in vitro* and in mice [161]. Furthermore, modulation of autophagy may enhance the effectiveness of CRC treatments. In the case of monoclonal antibody therapy targeting EGFR, anti-EGFR therapy induces autophagy which likely promotes tumor resistance to the treatment. Blocking autophagy along with anti-EGFR treatment has been proposed to circumvent autophagy-dependent resistance to anti-EGFR therapy [162]. This highlights autophagy as a therapeutic target in CRC; however, as discussed, the context in which autophagy is inhibited is important for preventing tumorigenesis and progression and so treatments need to be highly specific for the given setting.

Autophagy in intestinal manifestations of cystic fibrosis

Cystic fibrosis (CF) is a genetic disease caused by mutations in the gene, *CFTR* (cystic fibrosis transmembrane conductance regulator) [142]. The CFTR is a chloride channel at the apical membrane of epithelial cells [142]. The most severe CF pathology is airway obstruction due to viscous mucus and chronic inflammation leading to recurrent bacterial infections [142]. CF also presents with intestinal manifestations, including intestinal inflammation, obstruction, and a loss of tolerance to dietary antigens by immune cells, similar to that observed in celiac disease [142]. In mice, defective CFTR in IECs leads to decreased autophagy through TGM2 (transglutaminase 2)-mediated reduction of BECN1 [142]. Treatment of CFTR mutant mice with cysteamine, a TGM2 inhibitor, restores BECN1 and decreases the immune response to dietary antigens [142]. Cysteamine has also been shown to restore plasma membrane expression and function of a common CFTR mutation, F508del-CFTR, in mice and in patients through autophagy activation [143]. Together, these findings suggest a critical role for autophagy in CF pathogenesis and treatment that is largely underexplored. Insights into autophagy's involvement in oral tolerance may provide additional clues as to how autophagy is protective from intestinal manifestations of CF.

Conclusions

Great strides have been made in our understanding of autophagy within IECs both in a healthy and diseased context. While we can appreciate its role within ISCs and the two major secretory cell types, Paneth and goblet cells, more remains to be discovered about the role of autophagy within other epithelial populations. Autophagy provides protection

from a range of stressors, notably ER stress [65,69,78], oxidative stress [76], and pathogen invasion through xenophagy [58,66,67,129,130]; a breakdown of this protection leads to increased susceptibility to damage and impaired intestinal regeneration [65,69,76,78]. Furthermore, the specific mechanisms of autophagy-protected cytokine-mediated cell death within IECs during injury are not yet clearly understood. Cell types may be affected differently and there is a potential effect on IEC regeneration. Additionally, a heavy focus in the field has been laid on studying autophagy–deficient Paneth cells [63,68-70,137], and less is known about how autophagy may directly affect ISC metabolism and regeneration.

To add to the complexity, environmental factors have been identified to modulate Paneth cell autophagy and function. For instance, vitamin D deficiency has been demonstrated to decrease ATG16L1 levels, thereby decreasing lysozyme staining and Paneth cell numbers [163]. Additionally, metabolic dysregulation caused by smoking also modulates Paneth cell morphology and crypt apoptosis by interacting with the *ATG16L1*^{T300A} genotype and decreasing PPARG (peroxisome proliferator activated receptor gamma) signaling, both in humans with CD and in mice [164]. Further exploration of the interplay between environmental, dietary, infectious, microbial, and genetic factors is warranted.

Understanding the various ways in which autophagy affects intestinal epithelial homeostasis and recovery leads to a better comprehension of intestinal disease etiology, especially regarding IBD, opening avenues for the development of better and tailored treatments that are critically lacking.

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