Cell and Tissue Research (2024) 396:157–175
https://doi.org/10.1007/s00441-024-03894-7

REVIEW

Blood-testis barrier: a review on regulators in maintaining cell junction integrity between Sertoli cells

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Received: 23 August 2023 / Accepted: 20 March 2024 / Published online: 2 April 2024

Abstract

The blood-testis barrier (BTB) is formed adjacent to the seminiferous basement membrane. It is a distinct ultrastructure, partitioning testicular seminiferous epithelium into apical (adluminal) and basal compartments. It plays a vital role in developing and maturing spermatocytes into spermatozoa via reorganizing its structure. This enables the transportation of preleptotene spermatocytes across the BTB, from basal to adluminal compartments in the seminiferous tubules. Several bioactive peptides and biomolecules secreted by testicular cells regulate the BTB function and support spermatogenesis. These peptides activate various downstream signaling proteins and can also be the target themself, which could improve the diffusion of drugs across the BTB. The gap junction (GJ) and its coexisting junctions at the BTB maintain the immunological barrier integrity and can be the "gateway" during spermatocyte transition. These junctions are the possible route for toxicant entry, causing male reproductive dysfunction. Herein, we summarize the detailed mechanism of all the regulators playing an essential role in the maintenance of the BTB, which will help researchers to understand and find targets for drug delivery inside the testis.

Keywords BTB · Regulators · Proteins · Targets · Spermatogenesis

Introduction

Studies from the early twentieth century gave rise to the blood-tissue barrier concept. Dye was injected into whole animals, and it was observed that most of the tissues became stained, with the significant exceptions of the brain and testis (Setchell 2008). Through this observation, the blood-testis barrier (BTB) (Stanton 2016) and blood–brain barrier (BBB) concepts were developed (Banks 2019; Sweeney et al. 2019). Since these barriers make it challenging to find effective treatments for a range of ailments, the biological function of blood-tissue barriers has drawn more attention - particularly in their regulation.

The BTB forms between the ages of 12–14 years in human mammalian testis (Cheng and Mruk 2010b) and in rats by 17–21 days postpartum (Toyama et al. 2001). The BTB establishes a favorable niche required for spermatogenesis and creates a unique microenvironment for the development and maturation of germ cells (GCs) (Cheng and Mruk 2012). An essential function of the testis is spermatogenesis, carried out within the seminiferous tubules. Millions of sperm are generated daily through spermatogenesis (Cheng and Mruk 2012). In mammals, mitosis and meiosis is a central event in spermatogenesis. During these central events, preleptotene spermatocytes cross the BTB at the basal compartment, which involves transient openings of the basal ectoplasmic specialization (ES) and tight junctions (TJs). The GCs migrate toward the adluminal compartment via extensively changing shape (Yan et al. 2007). Remarkably, all the processes are linked with spermiation, an essential step in spermatogenesis. Spermiation occurs at the apical compartment, where matured spermatids or spermatozoa enter the tubule lumen and migrate to the epididymis for further development (Russell 1977). This process involves transient disruption of the apical ES and apical tubulobulbar complex. All of the spermatogenesis events occur at the opposite end of adjacent sertoli cells (SCs) in the seminiferous epithelium. Based on this, we can speculate that SCs coordinate between basal and apical components (Yan et al. 2007).

The cytoskeleton is comprised of significant proteins such as microtubules (MT), actin, and intermediate filaments. These

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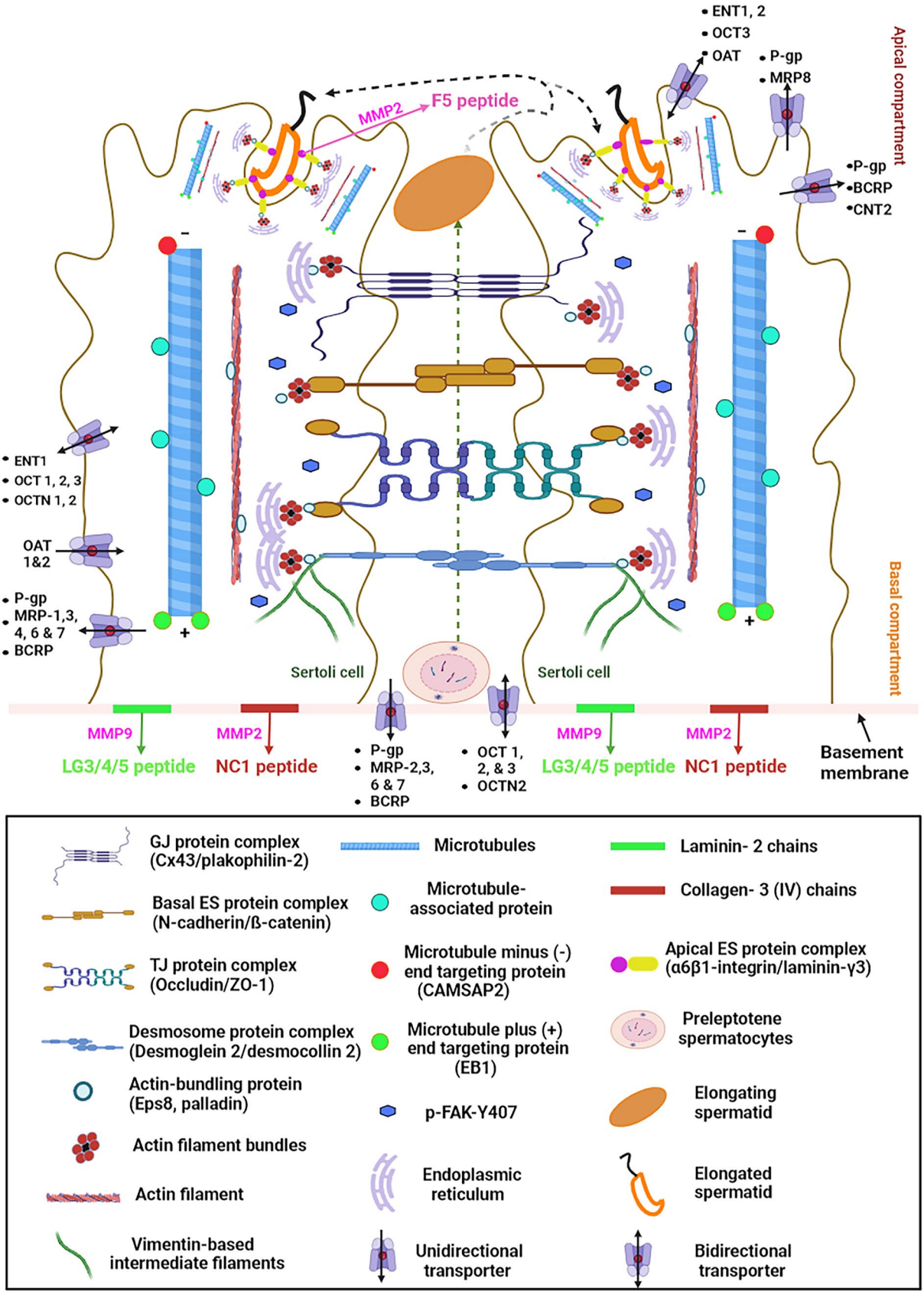
• ENT1, 2
• OCT3
• OAT
• P-gp
• MRP8
Apical compartment
MMP2
F5 peptide
•P-gp
•BCRP
•CNT2
• ENT1
• OCT 1, 2, 3
• OCTN 1, 2
OAT 1&2
• P-gp
• MRP-1,3, 4, 6 & 7
• BCRP
Sertoli cell
Sertoli cell
Basal compartment
MMP9
MMP2
MMP9
MMP2
LG3/4/5 peptide
NC1 peptide
• P-gp
• MRP-2,3, 6 & 7
• BCRP
• OCT 1, 2, & 3
• OCTN2
LG3/4/5 peptide
NC1 peptide
Basement membrane
GJ protein complex (Cx43/plakophilin-2)
Basal ES protein complex (N-cadherin/ß-catenin)
TJ protein complex (Occludin/ZO-1)
Desmosome protein complex (Desmoglein 2/desmocollin 2)
Actin-bundling protein (Eps8, palladin)
Actin filament bundles
Actin filament
Vimentin-based intermediate filaments
Microtubules
Microtubule-associated protein
Microtubule minus (-) end targeting protein (CAMSAP2)
Microtubule plus (+) end targeting protein (EB1)
p-FAK-Y407
Endoplasmic reticulum
Unidirectional transporter
Laminin- 2 chains
Collagen- 3 (IV) chains
Apical ES protein complex (α6β1-integrin/laminin-γ3)
Preleptotene spermatocytes
Elongating spermatid
Elongated spermatid
Bidirectional transporter

◂**Fig. 1** The morphology of BTB. This figure shows the compact structure of the BTB and transporters present at SCs and BM. Also depicted are various proteins that play a crucial role in regulating BTB integrity

proteins are prominent and functionally support the seminiferous tubule epithelium (Stanton 2016; Vogl et al. 2008). These cytoskeleton proteins in the BTB are exceptionally vulnerable to environmental toxins (Pineau 2020; Wan et al. 2013), such as heavy metals (Anyanwu and Orisakwe 2020; Venditti et al. 2021), plasticizers (Yao et al. 2010), and surfactants (Li et al. 2016; Wan et al. 2014). When the BTB is disrupted, it leads to a defect in spermatogenesis, which causes infertility in males. Thus, a better understanding of BTB regulators could potentially result in the development of improved therapies to handle toxicant-induced male reproductive disorders, as demonstrated in recent research (Li et al. 2016; Wan et al. 2014). Cadmium (Cd) transiently induces transforming growth factor-β2 (TGF-β2) and TGF-β3 which activates p38 mitogen-activated protein kinase (p38-MAPK) (Lui et al. 2003b; Wong et al. 2004) and induces BTB disruption in rodents (Hew et al. 1993; Wong et al. 2004). Thus, a p38 MAPK specific inhibitor can inhibit the Cd-induced BTB damage (Hew et al. 1993; Lui et al. 2003b). More specifically, to promote GC exfoliation, the BTB prevents male contraceptives like adjudin from influencing the apical ES between SCs and developing spermatids (Cheng 2014). Due to this, adjudin bioavailability is decreased. A high adjudin dose is needed to overcome Cd-induced male infertility. To increase adjudin bioavailability, it could be administered with BTB remodeling agents or proteins using magnetic-based nanoparticles in the testis (Chen et al. 2016).

Recent research has revealed that ES contains proteins like vinculin, paxillin, c-Src, and integrins; usually, these proteins are restricted to focal contacts (Siu et al. 2003b). More importantly, studies have demonstrated that BTB disruption induced by cytokines like tumor necrosis factor-α (TNF-α) and TGF-β3 or environmental toxicants, including Cd can be controlled by using particular ERK, JNK, or p38 MAPK inhibitors (Wong and Cheng 2005), suggesting focal adhesion kinase (FAK) as a potential upstream modulator of these MAP kinases (Siu et al. 2009a). An interruption in the BTB remodeling events prevents spermatogenic cells from migrating and orienting appropriately. It also causes a release of premature GCs from the epithelium, which results in sterility. Therefore, an in-depth understanding of regulatory factors between SCs and GCs during spermatogenesis at the BTB is needed for male reproductive physiology.

BTB's current concept and morphology

The BTB formed between the adjacent SCs is comprised of various protein junctions in the testis. The BTB is supported by basal ES (testis-specific actin-rich atypical adherens junction (AJ)) that provides a powerful cell adhesive function (Mao et al. 2020; Vogl et al. 2008; Wong et al. 2008a) (Fig. 1).

The basement membrane (BM) thickness in mammalian testis is approximately 0.15 µm (Siu and Cheng 2004b). The BM is quite similar to the BM of all epithelia and endothelia in multicellular animals, which have a thin, dense sheet-like extracellular matrix (ECM) (Kelley et al. 2014; Morrissey and Sherwood 2015). BM is also a part of the composed endothelium of testicular blood capillaries and lymphatic vessels located just underneath the tunica albuginea. It also has remarkably diverse functions in the seminiferous tubule of the testis (Liu et al. 2021). Conducting functional research to comprehend the biology and regulation of BTB dynamics requires a thorough understanding of its composition. In rodent testis, the BTB is currently understood to be composed of integral membrane proteins in nearby SCs (Cheng and Mruk 2002; Wong and Cheng 2005). The BTB is situated near the BM constituted by TJs, including AJ (basal ES, N-cadherin/β-catenin; nectin–afadin), JAM-A-zonula occludens-1 (ZO-1), claudin-ZO-1, macula adherens (desmosome, desmoglein-2-desmocollin-2), GJ (connexin 43/plakophilin-2), and occludin-ZO-1 (Cheng and Mruk 2009a; Lie et al. 2011) (Fig. 1). Thus, BTB divides the seminiferous tubule into two compartments known as apical and basal compartments (Cheng and Mruk 2012; Stanton 2016). Both compartments are similar, whereas apical ES is found in the adluminal section. The adluminal region has actin filament bundles due to the absence of notable structures on the heads of elongated spermatids (Vogl et al. 2008; Wong et al. 2008a) (Fig. 1). The physiological dynamics of the BTB are controlled by junction protein turnover and interactions (Yan et al. 2008a), which are subject to disruption or reorganization when exposed to exogenous toxins and pollutants (Cao et al. 2017; Tao et al. 2019). It has been discovered that abnormalities of the intracellular environment and harm to proteins such as connexin-43 (Cx43), claudin-5, and 11, occludin, and ZO-1 in the SC junction complex are the primary causes of the BTB destruction, which has a direct effect on spermatogenesis and results in infertility (Gao et al. 2015; Wang et al. 2013) (Fig. 2).

The BTB establishes an immunological barrier that isolates antigens from the systemic circulation to avoid the formation of anti-sperm antibodies in the testis, which emerge briefly during spermiogenesis. The ultrastructure of BTB, referred to as its function, imparts cell polarity and plays a significant role in determining the substances that have access to the growing spermatids in the apical compartment, such as ions, electrolytes, nutrients, hormones, paracrine, and autocrine substances (Cheng and Mruk 2010b; Wong and Cheng 2009). According to studies, the BTB is a highly dynamic ultrastructure that goes through significant remodeling from stages VIII to IX of the epithelial cycle to make preleptotene spermatocytes

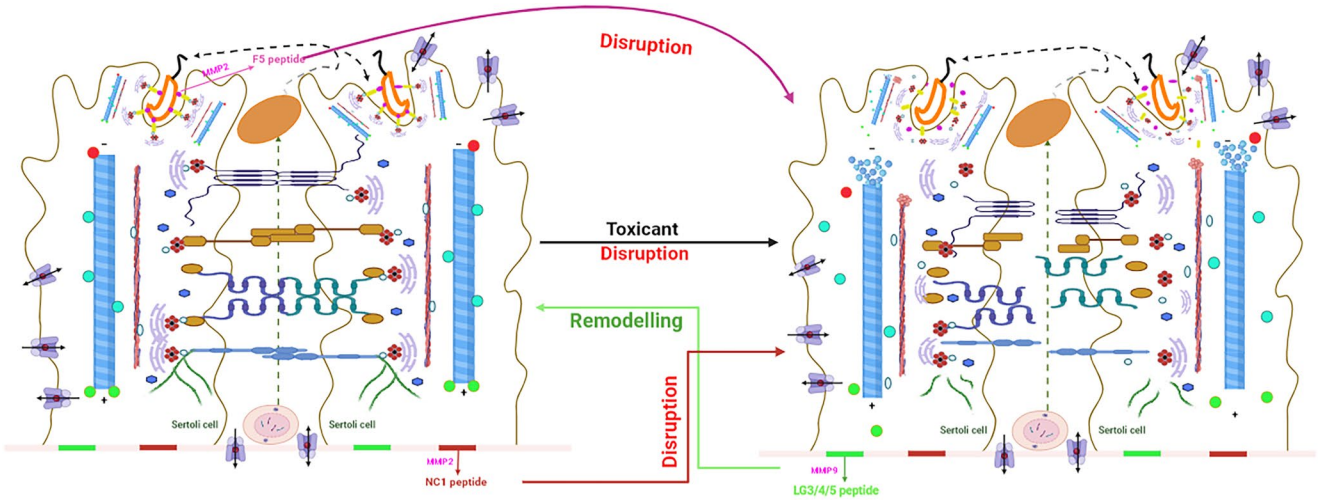

Fig. 2 Regulatory mechanism of testicular proteins in the destruction and reconstruction of BTB

more easily pass through the BTB (Cheng and Mruk 2010b; Mruk and Cheng 2010). Thus, it is considered that the testis has a unique mechanism that imparts barrier function while also allowing BTB remodeling to enable primary spermatocytes to pass at stage VIII of the spermatogenic epithelial cycle. Several drug transporters are present in SCs, including influx and efflux pumps. These transporters regulate the drug delivery to the developing GCs behind the BTB in the apical compartment and structurally link at the BTB with TJ protein complexes (Su et al. 2009) (Fig. 1).

BTB drug transporters

SCs express several drug transporters, whereas GCs are expressed during the spermatogenic seminiferous epithelial cycle at different stages in the mammalian testis (Su et al. 2011). However, it acts as an immunological barrier, a "safeguard." It is also challenging for drugs (e.g., adjudin) delivered to the adluminal compartment of the testis to exert its effects (Cheng and Mruk 2010a). The influx and efflux pumps play a role in determining the drug or harmful substance concentration in the apical seminiferous epithelium (Rochat 2009).

A drug must use the transporters to cross the biological membranes and reach its targets. There are membrane transporters like solute carrier transporters (SLCs) and ATP-binding cassette transporters (ABCs) (Su et al. 2011). Moreover, the basal membrane creates a transepithelial transport pathway, and apicolateral membrane efflux transporters may support these transport mechanisms (Klein et al. 2013). The drug must diffuse between cells or cross the membrane with any transport mechanism to cross the BTB. Some of the common drug influx transporters are equilibrated nucleoside transporters (ENTs) and concentrative nucleoside transporters (CNTs) (Klein et al. 2013), organic cation and anion transporters (OCTs and OATs) (Pizzagalli et al. 2002; Suzuki et al. 2003), and OATs transporting polypeptides are bidirectional transporters (Bakos et al. 2022; Huber et al. 2007) (Fig. 1). Some major efflux transporters at SCs basal membranes and in many tissues pump drugs back into the blood, including P-glycoprotein (P-gp) and a multidrug resistance-associated protein (MRP). These are linked with one of the BTB integral membrane proteins, including claudin-11, JAM-A, and occludin (Su et al. 2009), and breast cancer resistance protein (BCRP) (Bart et al. 2004; Hau et al. 2022; Siu et al. 2003b) (Fig. 1). MRP1 and P-gp are located in many testicular cells on the plasma membranes, including SCs, GCs, and myoid cells (Cheng and Mruk 2012; Huang et al. 2016). P-gp is one of the efflux drug transporters that localises at the blood-tissue borders and actively pumps therapeutic drugs out of the brain and testis (Abdul Razzak et al. 2019; Cheng and Mruk 2012; Erickson and Banks 2018) (Fig. 1). Together, these transporters actively pump drugs out of the testicles. MRPs are ATP-dependent efflux transporters that significantly transport a wide range of primarily anionic molecules. Furthermore, MRP3, 6, and 7 are the first line of defense against testicular toxicants such as PMCs in the human testis. This transportation or localization at the BM of SCs has a protective role at the BTB (Hau et al. 2022; Klaassen and Aleksunes 2010; Klein et al. 2014; Su et al. 2009, 2011).

ENT1 and ENT2 are present in SCs at the BM and apicolateral membrane in the testis (rat and human) (Klein et al. 2013). ENT1 drives the movement of nucleosides and their analogs in a bidirectional way, such as from SCs to blood or from blood into the SCs, and ENT2 permits the movement of these molecules from SCs into, or recycled from seminiferous tubule lumen (Hau et al. 2023) (Fig. 1). CNT2 is

present at the SCs apicolateral membrane, where it may play a role in delivering nucleosides into the adluminal compartment (Fig. 1). The function of CNTs is well-defined due to their affinities towards purine or pyrimidine nucleosides/bases and their uridine transport inhibition (Hau et al. 2022). OCTs have three types: OCT1, 2, and 3 regulate the diffusion of small cationic compounds, such as metformin, cisplatin, serotonin, dopamine, and choline, down their electrochemical gradients (Hau et al. 2022, 2023; Roth et al. 2012). OATs transporting polypeptides may play a critical role in hormonal transport to regulate spermatogenesis across the SCs membrane. OATs transporting polypeptide 4A1 and 1C1 are highly expressed in Leydig cells (LCs) in the testis (Hau et al. 2022; Pizzagalli et al. 2002) (Fig. 1). These can regulate hormone signaling and maintain testis homeostasis (Fujiwara et al. 2001). Overall, it can say that SCs can protect the development of GCs through efflux transporter expression on the basal membrane, which also helps to restrict therapeutic drugs from crossing BTB. Thus, study of BTB transporters, and regulatory proteins and molecules is crucial to developing mechanisms for safe drug delivery to the testis.

Regulators of BTB integrity

Researchers have focussed on endogenous protein regulators and their role in BTB integrity in the last couple of years. They found several biologically active protein-generated peptides via proteolytic cleavage by matrix metalloproteinases (MMPs) at the apical and basal compartments of the testicular seminiferous epithelium. This can effectively modulate the BTB function via downstream signaling proteins (Cheng and Mruk 2012). The F5 (Gao et al. 2016; Su et al. 2012) and NC1 (Chen et al. 2017; Wong and Cheng 2013) peptides are derived from laminin-γ3 (Yan et al. 2008b) and collagenous (IV) chain proteins and play a role in BTB disruption or remodeling. At the same time, the LG3/4/5 peptide domain is generated from the laminin-α2 chain protein. It maintains the integrity of the BTB (Mao et al. 2020; Siu and Cheng 2004a; Wu et al. 2020) (Figs. 2 and 3). Several downstream molecules, junction proteins, cytokines, and signaling pathways also play a vital role in BTB integrity. If these proteins and peptide mechanisms were fully discovered, we anticipated it would offer significant perspectives for a deeper comprehension of the molecular mechanism controlling BTB dynamics throughout the spermatogenesis epithelial cycle. Here, we have endeavored to describe all BTB regulators and their roles. We hope that researchers will use this review to thoroughly understand the physiological mechanisms of BTB regulators and identify targets for future therapies that maintain BTB integrity.

Peptides

F-5 peptide

The laminin-γ3 (LAMC3) protein is secreted by elongated spermatids in the apical compartment. This protein undergoes proteolytic cleavage by MMP2 and generates the F5 peptide domain, which has a significant role in BTB disorganization. It exerts its effect by varying its distribution across the seminiferous epithelium and downregulating the activated FAK (p-FAK-Y407) in the testis (Gao et al. 2016; Su et al. 2012) (Fig. 2).

The laminin-γ3 chain forms a trimeric protein with analogs like laminin-α3 and -β3 chains known as laminin-333 (Yan and Cheng 2006). This trimeric protein is a ligand to α6β1-integrin and forms a quadra complex called a *bona fide* complex (Siu and Cheng 2004c). This complex helps the attachment of spermatids onto SCs and supports spermatogenesis via conferring adhesion at apical ES (Wong et al. 2008a). However, during spermiation, this complex is deconstructed via MMP2-induced proteolytic cleavage. This leads to the release of F5 peptide from the IV domain of the laminin-γ3 chain (Siu and Cheng 2004c; Su et al. 2012), which downregulates different FAK active phosphomimetic mutants like p-FAK-Y407E. Changes in p-FAK-Tyr-407 expression in seminiferous epithelium activate neuronal Wiskott-Aldrich syndrome protein (N-WASP) (Gao et al. 2016; Lie et al. 2012; Su et al. 2012). Thus, based on the synergistic effects of N-WASP and actin-related protein (Arp3) combined with Eps8 protein, which provides plasticity to F-actin for reorganizing actin filaments across SCs in the testis and perturbating BTB permeability with spermatid exfoliation (Gao et al. 2016). Such filament change leads to BTB permeability and spermatid exfoliation (Gao et al. 2016; Su et al. 2012) (Fig. 3).

Non-collagenous (NC1) peptide

Studies have shown collagen I to XXIX subtypes in the mammalian tissues. Multiple genetic disorders can be found in humans in response to mutation or changes in many of these genes (Bateman et al. 2009; Ricard-Blum 2011). Among the 29 collagen subtypes, type IV collagen is a predominant structural component in the BM of the mammalian testis and in the BM of the glomerulus in the kidney (Miner 2012; Prockop and Kivirikko 1995; Siu and Cheng 2004b). Collagen α3 (IV) is the most prevalent subtype in the testicular BM among genetically distinct α1–α6 chains. Three α chains are formed into a triple helical structure that serves as the foundation for the collagen network. Each triple helical structure has 28 kDa and approximately 230 amino acid residues containing a C-terminal NC1 domain. Its cross-linking forms dimers

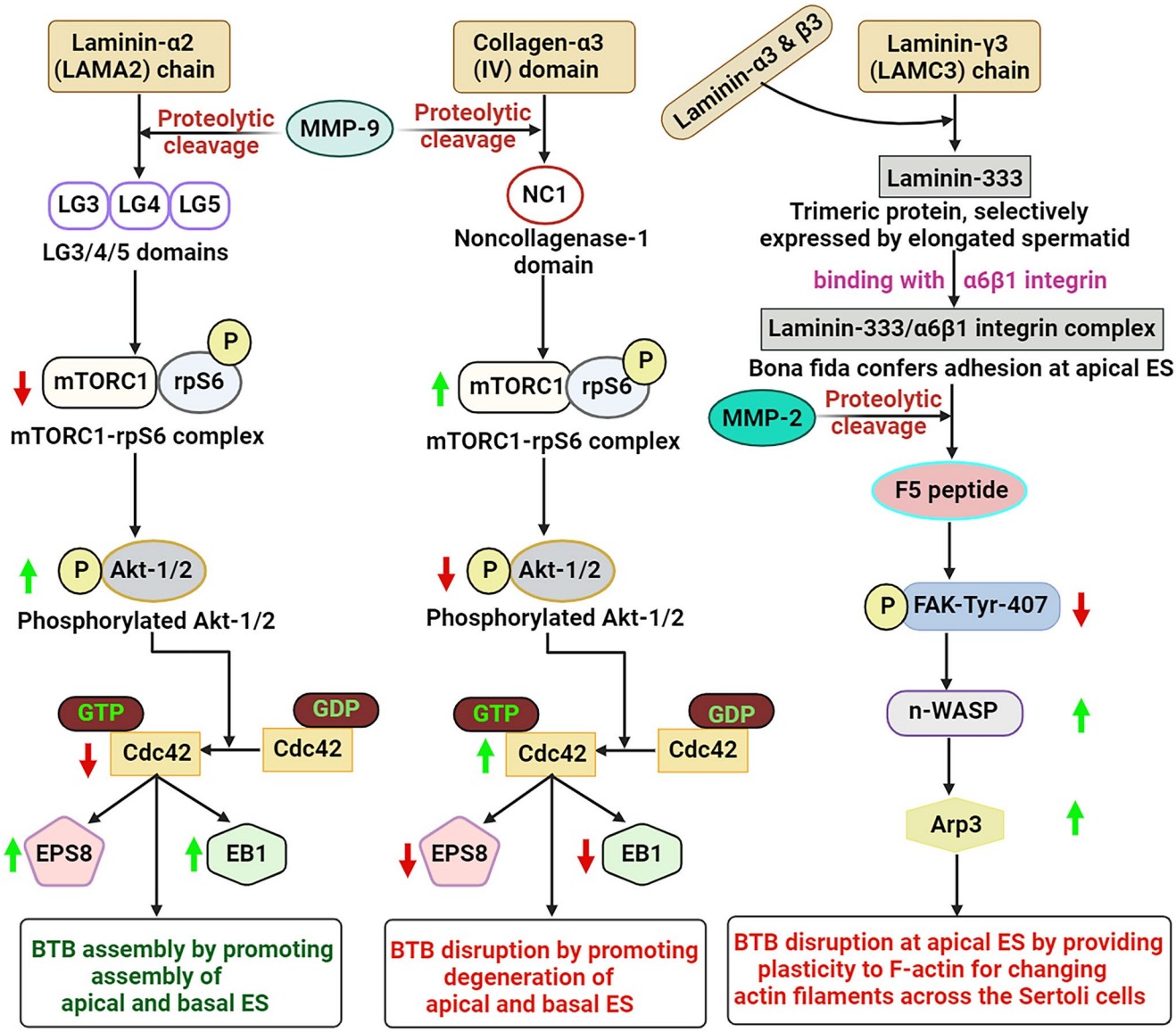

Fig. 3 A comparative regulatory mechanism of three main testicular proteins in remodeling and stabilizing the BTB integrity. (Abbreviations: LG3/4/5, Laminin-type globular 3/4/5 domains; NC1, Non-collagenase-1 peptide domain; MMP, Matrix metalloproteinase; FAK-Tyr-407, Phosphorylated-FAK on tyrosine residue; n-WASP, neuronal Wiskott-Aldrich syndrome protein; Arp3, Actin-related protein-3; Cdc42, Cell division control protein-42; EPS8, Epidermal growth factor receptor pathway substrate-8; EB1, End binding protein-1; ES, Ectoplasmic specialization.)

by using the 7S NC domain (Añazco et al. 2016) at the C-terminal or at the N-terminal, which is crucial to form collagen IV networks or to create a supra structure to sustain the BM (Ortega and Werb 2002; Risteli et al. 1980; Wong and Cheng 2013).

Testicular cells secrete collagen type IV chain containing an NC1 peptide domain (Rebustini et al. 2009). To support spermatogenesis, the BM of the testis generates bioactive collagen fragments to modulate cellular function in the seminiferous epithelium (Su and Cheng 2019). NC1 is a second bioactive peptide domain generated via proteolytic cleavage by MMP9 (Siu et al. 2003a) at the C-terminal region of the collagen-α3 (IV) chain (Chen et al. 2017; Wong and Cheng 2013) – a major constituent BM protein in seminiferous tubules (Siu and Cheng 2004a) (Fig. 2). Several in vitro and in vivo studies have shown that NC1 peptide induces basal ES/BTB remodeling (Chen et al. 2017; Su and Cheng 2019; Wong and Cheng 2013). Recently, Su et al. found that except RhoA, downstream mTORC1, rpS6, Akt1/2, and cell division control protein 42 homolog (Cdc42) are

activated by NC1-peptide, which induces BTB remodeling (Su and Cheng 2019). In this series of pathways, a small GTPase molecule, known as Cdc42, exerts its regulatory effects through cytoskeleton regulatory proteins that are actin and MT55 cytoskeletons (Pichaud et al. 2019; Su and Cheng 2019; Uehara et al. 2018) (Fig. 3). It is confirmed that the NC1 peptide has a crucial role in spermatogenesis regulation by activating mTORC1/rpS6/Akt1/2 and Cdc42 signaling (Liu et al. 2021). Furthermore, activated Cdc42 in the epithelium of SCs is associated with the significant downregulation of actin barbed end capping, EB1 (a microtubule plus [+] end tracking protein [+TIP]) and bundling protein Eps8 expression (Liu et al. 2020; Su and Cheng 2019) (Figs. 2 and 3).

LG3/4/5 peptide

The Laminin α2 (LAMA2) chain (formerly called merosin) has N- and C-terminal domains (Gao et al. 2017b). The 4a and 4b domains are present at the N-terminal of laminin, whereas LG1 to LG5 domains are present at the C-terminal. The N-terminal domains are situated between the long laminin coiled-coil (LCC) domain and laminin EGF-like domains, such as LEa, b, and c (Gao et al. 2017b; Wu et al. 2022). The C-terminal LG3/4/5 domains are 80 kDa fragments, which are generated via proteolytical cleavage of the LAMA2 chain by MMP9 at BM and transported to apical ES through an MT-dependent transport mechanism to exert its effects (Gao et al. 2017b; Li et al. 2020a). The LG3/4/5 peptide has the opposite effect of F5 and NC1 peptides, enhancing the BTB integrity (Gao et al. 2017b) (Figs. 2 and 3). An in vitro RNAi study found that the TJ barrier was disrupted upon LAMA2 chain knockdown (KD) in SC epithelium (Gao et al. 2017b) and also found high expression of two activated forms of rpS6 as p-rpS6-S235/S236 and p-rpS6-S240/S244 (Gao et al. 2017a). Thus, the researchers asserted that the LG3/4/5 peptide exerts its downstream effects similarly to the NC1 peptide with opposite action via the mTORC1/rpS6/Akt1/2 signaling complex (Gao et al. 2017a) (Fig. 3). It illustrates the regulatory mechanism consisting of a local physiological axis where C-terminal laminin domains act as an autocrine peptide, namely LG3/4/5 fragment between the BM and the BTB (Gao et al. 2017a, b). LG3/4/5 fragment is a unique peptide in the apical ES BTB-BM regulation. This fragment works in concert with the F5 peptide to support the epithelial cycle of spermatogenesis. In short, LG3/4/5 fragments have protective effects in maintaining BTB integrity in the testis (Bu et al. 2022; Li et al. 2021). It also revers the Cd-induced BTB disruption and testicular injury and prevents male reproductive dysfunction, showing its therapeutic potential to treat male infertility (Li et al. 2020b) (Fig. 2).

Mammalian target of rapamycin complex 1 and 2 (mTORC1 and mTORC2) and ribosomal protein S6 (rpS6)

NC1 peptide activates downstream signaling protein targets such as mTORC1 (Condon and Sabatini 2019; Laplante and Sabatini 2012) and rpS6 (Meyuhas 2015) and inactivates Akt1/2 (also known as protein kinase B (Shariati and Meric-Bernstam 2019)). Further downstream, it activates Cdc42 and remodels F-actin and MT organization (Su and Cheng 2019) (Fig. 3). Studies have reported that the mTORC1 signaling protein is a combination of mTOR (a Ser/Thr non-receptor protein kinase) with its binding partner Raptor (regulatory-associated protein of mTOR) (Shimobayashi and Hall 2014). In vitro, these signaling proteins work with rpS6 to promote SCs BTB disruption (Mok et al. 2015). However, in vivo, it was found that phosphorylated rpS6 mutant type (p-rpS6-MT) increases BTB leakiness more than phosphorylated rpS6 wild type (p-rpS6-WT) through changes in the organization of F-actin and microtubule (MT)-based cytoskeletons regulatory proteins. This leads to spermatogenesis defects, including failure of spermatid and organelle transport and loss of spermatid polarity, followed by germ cell exfoliation (Li et al. 2018).

Studies revealed that mTORC1 and mTORC2 act as molecular switches, making the barrier "open/leaky" or "closed/tight"; these molecules have opposite effects on BTB integrity (Fig. 4). The regulation of the mTOR complexes supports preleptotene spermatocyte transportation across the immunological barrier via BTB remodeling (Li and Cheng 2016; Mok et al. 2013a) (Fig. 3). Rodent and in vitro studies have reported that mTORC1 is a downstream signaling protein (Shimobayashi and Hall 2014). It mainly targets rpS6 and promotes SC BTB disassembly (Mok et al. 2014, 2015). This process happens when preleptotene spermatocytes cross the BTB at stage VIII of the epithelial cycle (Xiao et al. 2014), whereas mTORC2 makes the BTB tighter (Mok et al. 2013b).

Due to their antagonist effects, these two signaling proteins are effectively expressed in mammalian SCs and GCs in the seminiferous tubule and work simultaneously. mTORC1 dissociates the BTB above the preleptotene spermatocytes during stage VIII of the epithelial cycle of spermatogenesis, whereas mTORC2 triggers the assembly of BTB behind it. This coordination seals the BTB or immunological barrier (Cheng and Mruk 2012; Su et al. 2013) (Fig. 4). In this context, in vivo (Hew et al. 1993) and in vitro (Siu et al. 2009b) studies have shown that $CdCl_2$ induces BTB disruption and also induces disruption of cytoskeletal filaments in the testis. ROS production and accumulation contribute to mTORC1 and mTORC2 regulation imbalance. Meanwhile, ROS triggers the mTORC1 activity (Mroueh et al. 2019) and decreases the activity of mTORC2 (Rodriguez-Vargas et al. 2020). Overall, mTORC1/rpS6 signaling disrupts

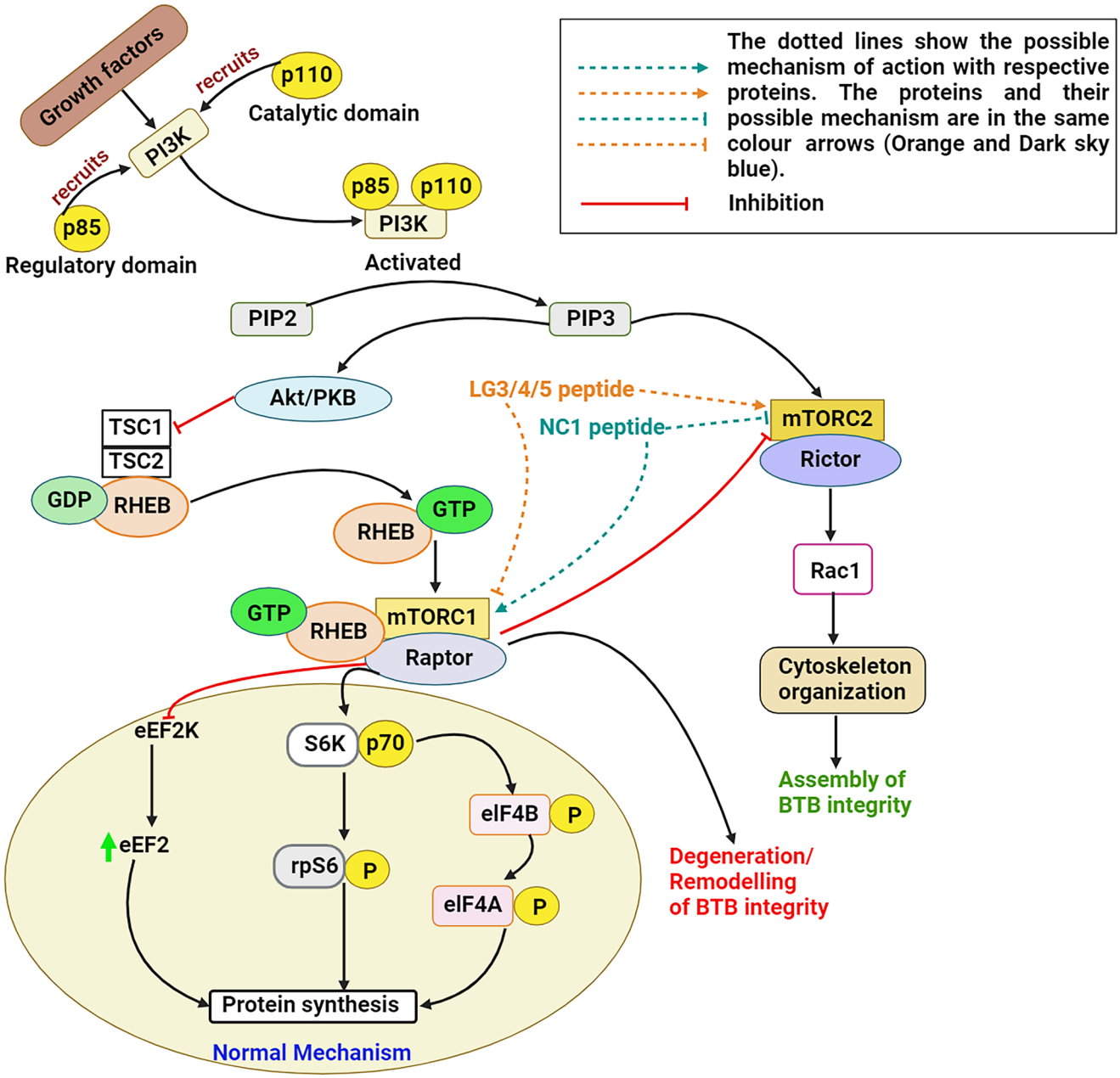

Fig. 4 A role of mTORC1 and mTORC2 as a molecular switch in regulating BTB integrity. If LG3/4/5 inhibits mTORC1, the mTORC2 will activate and make the BTB assembly. Whereas, in action, to NC1 peptide, it will reverse. This figure illustrates the normal mechanism of mTORC1 and the possible mechanism of testicular proteins in interaction with mTORC1 and mTORC2 molecules. (The yellow oval shape states a normal mechanism of involvement of mTORC1 regulation in protein synthesis with coordination of several elongation factors and ribosomal proteins)

the BTB integrity through Akt/MMP9, and mTORC2/Rictor signaling promotes BTB integrity via PKCα/Rac1 (Mok et al. 2013b, 2014; Wei et al. 2021).

A-kinase anchoring protein-9 (AKAP9)

AKAP9 is a 450-kDa signal-organizing scaffold protein known as AKAP450 or CG-NAP. AKAP9 binds to nucleotide phosphodiesterase PDE4D3 and reduces cAMP levels in the centrosomes in SCs (Taskén et al. 2001). A deficiency in cAMP-responsive binding partners has been shown to contribute to defects in GC-SC interactions (Aivatiadou et al. 2007) or sperm motility (Skålhegg et al. 2002), resulting in male infertility. AKAPs bind protein kinase A (PKA) (Pidoux and Taskén, 2010), and in some situations, the cAMP-responsive guanine exchange factor Epac1 (Sehrawat et al.

2011). AKAP9 KD in mice leads to extensive disorganization of actin and MT-based cytoskeletons at the BTB, causing male infertility (Venkatesh et al. 2016). AKAP9 recruits other functional proteins to interact with the Golgi and centrosome, forming an AKAP9/Golgi/Centrosome. This complex facilitates γ-tubulin ring complex assembly, which is necessary to initiate MT nucleation and anchorage (Wang et al. 2020; Wu et al. 2021). AKAP450, a longer isoform, confers MT-nucleation at the Golgi (Rivero et al. 2009) and regulates MT dynamics (Sehrawat et al. 2011) via the MT+end dynamics regulators, i.e., end-binding proteins, including EB1 (Jiang and Akhmanova 2011). When AKAP9 is KD in endothelial cells, EB1 expression decreases, ultimately affecting the MT polymerization rate and Epac1/2-stimulated MT growth (Sehrawat et al. 2011). AKAP9 KD also prevents barrier function in endothelial cells (Oldenburger et al. 2014; Sehrawat et al. 2011). A study by Venkatesh et al. found that AKAP9 deletion leads to infertility even after BTB formation at puberty. This AKAP9 deletion leads to changes in MT organization in SCs and loss of barrier integrity, specifically F-actin and BTB integrity proteins in the apical compartment (Venkatesh et al. 2016).

Hemidesmosome

Hemidesmosomes are the connecting filament found at the SC-BM interface in the mammalian testis and have various distinctive and crucial responsibilities at the BTB (Cheng and Mruk 2010b; Lie et al. 2010). The α2-laminin chain and β1-integrin appear to be the putative components of the hemidesmosome. It was reported that functional disruption of β1-integrin would affect the function of the TJ barrier. This shows the association between the BTB junction and hemidesmosome (Yan et al. 2008b). Another study reported that RNAi-mediated KD of desmoglein-2 and desmocollin-2 disturb the SC-TJ permeability and causes mis-localization of an integral membrane protein and a cell adhesion molecule at the BTB (Mirza et al. 2007; Wang et al. 2007). It was also found that CAR and β-catenin combinedly interact at the basal ES with N-cadherin-based AJ complex, moving from the cell-cell interface to cell cytosol. This increases the endocytosis of CAR and, thus, destabilizes cell adhesion function at BTB (Lie et al. 2010). Studies have shown that desmoglein-2 is associated with c-Src. The activity of c-Src is associated with CAR (Wang et al. 2007), Cx43 (Li et al. 2009), N-cadherin, occludin, and ZO-1, whereas desmoglein-2 did not interact with these BTB structural proteins (Lee and Cheng 2005). Desmosomal protein forms a complex at the BTB known as desmocollin-2/desmoglein-2/c-Src desmosomal protein complex. This complex may serve as a dominant regulator with c-Src, which can confer the proper phosphorylation status of BTB proteins. Besides this, it illustrates that desmosomes in interaction with c-Src serve as a signaling platform at the BTB (Cheng et al. 2011).

Gap junction (GJ) and polarity proteins

A GJ is a cell-cell actin-based communicating junction composed of two opposite connexons between adjacent SCs at the BTB. The hemichannel is the functional unit of the GJ within each connexon (Vinken et al. 2011). This channel allows diffusion of solutes, < 1–1.5 kDa in molecular mass; however, large molecular size substances, including siRNA duplexes, cyclic nucleotides, and polypeptides, can cross GJ with specific permeability (Harris 2007; Maeda and Tsukihara 2011). GJs are linked with TJs, desmosomes, and basal ES. KD studies of Cx43 reported that loss of Cx43 alone could not disrupt the function of the TJ barrier in SCs (Li et al. 2009). In some models, it was also found that Cx43 KD hinders the re-assembly of the disrupted TJ barrier. Thus, these findings illustrate that Cx43 plays a crucial role in established BTB reassembly or TJ barrier at the VIII stage of the spermatogenic cycle but not in new BTB TJ barrier assembly (Li et al. 2010).

The Crumbs protein complex [e.g., Crumbs-3/PALS1 (protein associated with (LIN-7)-1)/PATJ (PALS1-associated tight-junction protein)], PAR (partitioning defective) protein complex [e.g., PAR3/PAR6/αPKC (atypical protein kinase C (PKC), a nonreceptor Ser/Thr protein kinase)], and the Scribble complex [e.g., Scribble/LGL1/2 (Lethal giant larvae 1/2)/DLG1 (Discs large 1)] are the three modules of polarity proteins that have at least one homolog found in mammals (Li et al. 2010; Wong and Cheng 2009). These polarity proteins are involved in cell adhesion in the adluminal compartment at the SC-elongated spermatid interface and SC-SC interface at the BTB (Wong et al. 2008b). The study showed that polarity proteins, including PAR5 (14-3-3) and Cdc42, are involved in protein endocytosis at the SCs BTB. RNAi-mediated KD of 14-3-3 significantly increases endocytosis of JAM-A and N-cadherin (Wong et al. 2009). Additionally, high expression of dominant negative mutant Cdc42 in SCs inhibits TGFβ3-induced TJ barrier disruption (Wong et al. 2010). Overall, this illustrates that polarity proteins play a significant role in protein trafficking events at BTB (Cheng et al. 2011).

Ca^{2+}-mediated autophagy

Free Ca^{2+} is a vital secondary messenger or signaling molecule for the survival of all higher species (Harr and Distelhorst 2010). This messenger is involved in several pathophysiological processes in cells (Bootman et al. 2001), such as cell proliferation, hormone secretion, apoptosis, and

autophagy (Patergnani et al. 2020). When extracellular stimuli activate cells, Ca^{2+} is released from internal Ca^{2+} reserves or taken in from the extracellular environment. The high intracellular concentration of Ca^{2+} is due to the input of extracellular Ca^{2+} ions through the Na^{+}/Ca^{2+} exchanger or the voltage-gated ion channel (VGCC) (Berridge et al. 2003; Gurkoff et al. 2013; Kumar et al. 2014).

Several studies have reported that an increased intracellular free Ca^{2+} concentration is a leading cause of apoptosis (Bauer and Murphy 2020). Other studies show that Ca^{2+} has a positive regulatory effect on autophagy in normal conditions. In contrast, it also has a negative regulatory effect in stress conditions. Free Ca^{2+} ions promote autophagy through different pathways, including calmodulin-dependent protein kinase-β (CaMKK)–AMPK–mTOR pathway, beclin1 pathway, and IP3R pathway (Chen et al. 2012; Feng et al. 2020). It also inhibits autophagy through IP3R, BECLIN1-Bcl-2 complex, and AMPK-mTOR pathways (Cárdenas and Foskett 2012; Decuypere et al. 2011). There is little evidence of a relationship between Ca^{2+} and BTB disruption. Studies have reported that Ca^{2+} might be indirectly involved in TJ destruction through kinases such as PKC (Long et al. 2007) or p38-MAPK (Mu et al. 2008). A recent study by She et al. shows that an increased level of free Ca^{2+} ions in the cytoplasm with autophagy markers such as LC3-II and p62 in response to Zearalenone (ZEA)-induced BTB destruction (She et al. 2021).

C-type natriuretic peptide (CNP), TGF-β3 and TNF-α

Testicular gonadal peptide hormones exert their effects by coupling with cell surface guanylyl cyclase receptors (Potter and Hunter 2001), also known as natriuretic peptide (NP) receptors (NPR)-A and NPR-B. These receptors catalyze the synthesis of second messengers and control the intracellularly present amount of cGMP (Potter et al. 2006; Reubi 2003). The function of the endothelium barrier can be affected in various ways by different NPs. For instance, a cGMP-independent, perhaps Rho A-dependent mechanism enables ANP but not CNP to inhibit thrombin-induced collapse of the rat lung microvascular endothelial barrier (Klinger et al. 2006). Thus, ANP may work with CNP to control junction remodeling activities during spermatogenesis. In this respect, it has been indicated that TNF-α (Suga et al. 1993) and TGF-β3 (Suga et al. 1992) up-regulate the secretion of CNP in cultured aortic endothelial cells. A study by Xia et al. reported that CNP acts as a regulator of BTB dynamics. It is expressed stage-specifically and localized predominantly at the BTB. CNP may work synergistically with cytokines at the VIII stage of the spermatogenic epithelial cycle - preleptotene spermatocyte migration - to remodel the BTB and make it leaky. SCs and GCs form the seminiferous epithelium's pool of CNP. However, the NPR-B receptor was found nearly entirely in SCs (Xia et al. 2007).

SCs or GCs secret cytokines, namely TNF-α and TGF-β3 at the BTB microenvironment in the seminiferous epithelium that can induce reversible BTB disruption in vivo (Li et al. 2006). These cytokines play a vital role in 'restructuring' and/or 'opening' of the BTB to facilitate the preleptotene spermatocytes transport across the BTB during stage VIII of the epithelial cycle (Li et al. 2006; Xia et al. 2006), apparently by decreasing the expression of ZO-1 and occludin at the BTB via the p38-MAPK signaling pathway (Lui et al. 2001). TGF-β3 (Xia et al. 2006) or TNFα (M. W. Li et al. 2006) can reversibly disrupt BTB integrity in adult rat testis. BTB markers were analyzed using dual-labeled immunofluorescence for JAM-A, N-cadherin, occludin, and ZO-1 to check BTB disruption. More significantly, it was discovered that reversible BTB integrity disruption in the testis was caused by local administration of TNF-α at the same concentration as its endogenous level. The action of mechanisms or pathways followed by cytokines leading to decreased integral membrane proteins at BTB is still unknown (Xia et al. 2009).

FAK and its phosphorylated forms

FAK is a nonreceptor protein tyrosine kinase having 125 kDa in molecular weight. It is composed of a band 4.1, ezrin, radixin, moesin (FERM) domain and a focal adhesion targeting (FAT) domain located near the N-terminus and C-terminus, central catalytic kinase domain, and three proline-rich regions (PRI, PRII, PRIII) domains (Lim et al. 2008; Siu and Cheng 2004b). Furthermore, it contains FAK-Tyr-397 as an autophosphorylated site among all the six putative phosphorylation sites such as Tyr-397, -407, -576, -576 (Cheng and Mruk 2009b; Ilic et al. 1997), and is also recognized as a modulator of integrin-associated signaling pathway (Bouchard et al. 2008). FAK is a crucial regulator of TJs and AJs (Ozaki et al. 2007), often only found at the cell-extracellular matrix interface at the focal contact (Parsons 2003). It regulates cell differentiation, adhesion, cell motility/movement, cell cycle progression, death, and the TJ permeability barrier in diverse epithelia and/or endothelial cells (Broussard et al. 2008; Parsons et al. 2008). FAK is expressed in a stage-specific manner in the seminiferous epithelium at the BTB, and it forms a structural protein complex with occludin and ZO-1 (Siu et al. 2009a). Also, Siu et al. reported the conjugation of FAK with occludin but not with other TJ proteins, such as junctional adhesion molecule-A or claudin-11. FAK and occludin are co-localized in virtually all the stages of the seminiferous epithelial cycle at BTB. However, this co-localization is diminished during the transportation of primary leptotene spermatocytes across the BTB at the VIII–IX stages of spermatogenesis (Siu et al. 2009b). p-FAK-Y407 is one of the FAK-activated forms. It helps spermatogenesis by

changing BTB integrity via its extensive-expression at the apical and basal compartments in SCs and SCs adhesion at the corresponding site (Lie et al. 2012). Its high expression enhances spermatogenesis by increasing cell adhesion function. However, its mutant overexpression, namely p-FAK-Y407E, can rescue PFOS-induced SC injury in primary rat SC culture (Wan et al. 2014). Previously, two activated forms of FAK were reported, namely p-FAK-Tyr397 and p-FAK-Tyr576. These are localized to the apical compartment and are known as a component of apical ES (Siu et al. 2003b) in adult rat testis (Wong et al. 2008a). The p-FAK-Tyr397 is an autophosphorylated form of FAK. Subsequent studies have shown that it localized and interacted with α6β1-integrin mainly at the apical ES in the SC-ES interface (Beardsley et al. 2006; Siu et al. 2003b). α6β1-integrin forms a *bona fide* complex with the laminin-α3β3γ3 (Yan and Cheng 2006), which regulates adhesion between developing spermatids and SCs. At the apical ES, this complex remains until spermiation (Beardsley et al. 2006). A FAK KD study showed that SCs are desensitized to Cd, and the TJ barrier retains the same at BTB. Therefore, it was also reported as a molecular target of Cd (Siu et al. 2009a). However, it is still uncertain whether FAK at the BTB is activated at only Tyr-397 and Tyr-576 residues and whether it modulates occludin adhesion function at TJs of BTB (Beardsley et al. 2006; Siu et al. 2003b). In summary, FAK is a significant protein in maintaining BTB integrity and supporting spermatogenesis during BTB dynamics in the testis.

FYN

FYN, a non-receptor tyrosine kinase from the SRC family, makes it easier for viruses to pass epithelial TJs. There are two non-receptor tyrosine kinases belonging to the SRC family of kinases (SFKs) such as SRC and YES, having several palmitoylation states in their SH4 domain (Xiao et al. 2012, 2019). To maintain protein synthesis and homeostasis at the apical ES and the BTB, they perform various functions in intracellular protein trafficking activities in SCs that are distinct (Kasahara et al. 2007; Sato et al. 2009). Non-palmitoylated SRC is likely to mediate protein degradation at the "old" BTB and apical ES disassembly and SC phagocytosis of residual bodies. This SRC is rapidly transported between the plasma membrane and late endosomes or lysosomes (Kasahara et al. 2007; Sato et al. 2009).

FYN has been discovered to closely resemble the expression pattern of the TJ and basal ES at the BTB, as well as the expression pattern of actin-binding proteins such as Arp3, annexin A2, and Eps8 during postnatal testicular development and junctional restructuring caused by $CdCl_2$. As such, FYN may collaborate with several proteins, potentially modifying their activity and/or location through FYN-dependent tyrosine phosphorylation (Cheng and Mruk 2012; Xiao et al. 2019). In addition to FYN's well-known localization to the actin-based ES, a radial pattern of its distribution in the SC stalk raises the possibility of a close relationship between FYN and other cytoskeleton proteins seen in SCs (Maekawa et al. 2002). Particularly, FYN may connect with intermediate filaments of vimentin and/or microtubules, both of which localize along the SC stalk (Johnson 2014; Mruk and Cheng 2015). Studies suggest that FYN may be involved in GC transport through performing tasks including assembling and disassembling SC-GC junction, eliminating apical ES and cytoplasmic residues of spermatozoa, and phagocytosing leftover bodies in the seminiferous epithelium (Li et al. 2017; Xiao et al. 2014). FYN is phosphorylated and has an ES/desmosome protein (plakoglobin) interaction at the BTB of rat testes (Mruk et al. 2017). However, it is unclear how FYN functions in mammalian testes to maintain the BTB integrity and the adherence of GCs to SCs. It was determined that FYN shares structural similarities with the actin- and microtubule-based cytoskeleton structures. An interaction of FYN with Arp3 (branching or nucleation protein) and triggering the phosphorylation of Arp3 were linked to $CdCl_2$-induced epithelial restructuring, which may have resulted in actin cytoskeleton remodeling, BTB damage, and the loss of GCs in the seminiferous epithelium (Yang et al. 2022). Further studies are required to better understand the role of FYNs in the basal ES/BTB and apical ES compared to SRC or YES.

The NADPH oxidase 1 (NOX1)

NOX1 is a member of the NADPH oxidase family and is a major source of ROS responsible for microvascular dysfunction in metabolic disease (Muñoz et al. 2020; Thompson et al. 2017). A series of enzymatic reactions produce ROS in many cell compartments, including the cytoplasm, cell membrane, endoplasmic reticulum, mitochondria, and peroxisomes. Expression of antioxidative enzymes significantly decreased and ROS levels rose dramatically in response to glyphosate (GLY) exposure. Increased expression of NOX1, a significant member of the NOX family, was thought to cause an increase in ROS level. An earlier investigation established that NOXs generate O_2^- through NADPH electron exchange and that NOX-derived ROS generation impacts various metabolic functions and disease states (Forrester et al. 2018). Under normal conditions, NOX plays a crucial role in spermatogenesis by maintaining the control of intracellular ROS homeostasis (Bedard and Krause 2007). However, NOX-generated high ROS accumulation leads to oxidative stress, contributing to several diseases (Schwerd et al. 2018; Xu et al. 2014). Additionally, oxidative stress can be controlled by a specific NOX1 inhibitor, namely ML171, which lowers ROS generation and enhance antioxidant enzyme activity, including that of SOD and CAT (Shen

et al. 2016). Regarding the mechanism, Liu et al. showed that GLY directly interacts with ER- at the sites of Pro39 and Lys401 to encourage ER- activation, which increases NOX1 expression to cause ROS accumulation. According to studies by Liu et al. GLY's direct interactions with ER- at the Pro39 and Lys401 sites lead to ER- activation, which enhances NOX1 expression and results in the accumulation of ROS. Based on transcriptome analysis, they discovered a significant increase in the oxidative stress-related NOX1 gene in GLY-exposed testis. They found that the NOX1 gene expresses NADPH oxidase and triggers ROS overproduction, which is responsible for BTB disruption in the GLY-exposed testis. NOX1 KD reduced the GLY-induced loss of TJ proteins at BTB as well as decreased oxidative stress in SCs (Liu et al. 2022).

α_2-Macroglobulin via the c-Jun N-terminal protein kinase pathway

Earlier studies discovered that α2-Macroglobulin (α2-MG) is one of the protease inhibitors in the testis. It is a 720 kDa glycoprotein consisting of four similar 180-kDa subunits (Fritz 1993). An in vitro study reported α2-MG facilitates the attachment of GCs onto the SCs epithelium in concert with other proteases (Mruk et al. 1997, 2003). SCs produce α2-MG, which localizes to the SC-SC and SC-GC interface during $CdCl_2$-induced BTB disruption in adult rat testis. Its regulating mechanism is unknown (Wong et al. 2005). An in vivo study on $CdCl_2$-induced BTB junction damage examined the participation of α2-MG in BTB junction restructuring. Significantly, it was induced in the seminiferous epithelium and localized to SC-SC TJs, basal ES, and apical ES near the heads of detaching spermatids (Wong et al. 2004). These findings show that α2-MG has a central role in spermatogenesis by protecting its relevant junction restructuring from damage at the seminiferous epithelium. However, BTB junction proteins are regulated by TGF-β3 via the p38 MAPK pathway during $CdCl_2$-induced BTB disruption, not via the α2-MG pathway (Lui et al. 2003b; Wong et al. 2004). Hypothetically, α2-MG may have a role in extensive tissue remodeling in the seminiferous epithelium pertinent to spermatogenesis by controlling unwanted proteolysis (Zhu et al. 1994). In adrenocortical (Shi et al. 1990) and astrocytoma cells (Fabrizi et al. 1994), TGF-α and interferon-γ can stimulate α2-MG (Feige et al. 1996; Lui et al. 2003a; Siu and Cheng 2004b); whereas its relatively high level in the testis is maintained to protect the epithelium during tissue remodeling pertinent to spermatogenesis (Braghiroli et al. 1998; Li et al. 1994). α2-MG maintains cytokine levels and regulates junction remodeling in seminiferous epithelium via its interactions with a biological factor such as TGF-β3.

Conclusion and future perspective

According to recent findings, the BM of the adult rat testis produces several regulatory macromolecules. In this perspective, the three most well-known protein chains are formed locally in the testis. Proteolytic cleavage of collagen chains by MMP2 (at the apical ES) or structural laminin by MMP9 (at the BM) have been discussed. Other regulatory biomolecules are also highlighted, which may be the subject of future research on BTB remodeling and are potential targets for drug delivery to the testis; for example, rpS6 may become a therapeutic target to treat toxicant-induced testicle damage. However, in addition to the research data mentioned above, it is essential to identify whether integrin-based receptors are most likely the binding partners of F5, NC1, and LG3/4/5-peptides. Cytoskeleton proteins such as actin and MT in SCs are modulated by these physiologically active peptides and their downstream signaling proteins (other BTB regulators mentioned above), which in turn impacts cell adhesion in the seminiferous epithelium at the SC-GC contact (apical ES) and the SC-SC interface. These modifications may cause GC exfoliation and BTB remodeling, hindering or enhancing spermatogenesis. The molecules and/or mechanisms that regulate BTB regulation events are still not fully understood.

Based on the available literature, it can be hypothesized that if the mechanism of action of these proteins and peptides was fully explored or understood, researchers should focus on synthetic proteins or peptides based on discussed BTB regulators in this review, which will penetrate through the drug transporters at SCs BTB. If possible, a timely degrading activator or inhibitor could be a better option for manipulating the discussed BTB regulators and their intermediates for delivering a drug to the testis. For example, the F5-peptide and NC1-peptide could be activated to induce leakiness in the BTB, allowing for easier drug delivery. In the future, if possible, a delayed, degradable activator should be administered with the drug to target MMP2 and mTORC1/rpS6/Cdc42. The pathways will be activated regarding F5-peptide and NC1-peptide (shown in Figs. 2 and 3) and keep BTB open to deliver the drug. Once the drug enters the seminiferous tubule and the activator degrades, LG3/4/5 peptide activity will induce remodeling of the BTB. Simultaneously, the LG3/4/5 peptide should be inhibited, which will keep BTB leaky in response to other peptides such as F5 and NC1. Advanced studies on the regulation of these three peptides are needed to evaluate detailed mechanisms and possible strategies to deliver drugs into the seminiferous tubule via coordinated manipulation. As per the available literature, we can say that advanced studies are needed to better understand the mechanism of mTORC1, mTORC2, AKAP9, Hemidesmosomes proteins,

free Ca^{2+} ions levels, CNP with cytokines, FAK, FYN, GJ and Polarity proteins, NADPH oxidase 1 (NOX1), and α_2-Macroglobulin via the c-Jun N-Terminal protein kinase pathway in BTB regulation.

Here, we have suggested that the overexpression of therapeutic proteins and their downstream signaling molecules, or other factors that play a crucial role in BTB remodeling and supporting spermatogenesis, should be carefully evaluated as a therapeutic option to treat male infertility. Bioinformatic strategies will be helpful in future studies on BTB regulators. These analyses will help to identify the involved integrin receptors, which can be confirmed by biochemical analysis, and the downstream signaling proteins. These data will allow us new insights into the biology of spermatogenesis. In this review, we tried to highlight the specific area of research that needs to be the subject of additional research. In conclusion, this is an area of male reproductive biology that has received little attention and needs to be further investigated.

Acknowledgements The authors thank the VIT, Vellore, Tamil Nadu, India, for supporting this work.

Author contributions Conceptualization, U.R.W., A.V.G.; resources and data curation, U.R.W., A.V.G.; writing— original draft preparation, U.R.W., A.V.G.; writing—review and editing, U.R.W., A.V.G.; visualization, U.R.W., A.V.G.; supervision, U.R.W., A.V.G.; project administration, U.R.W., A.V.G., All authors have read and agreed to the published version of the manuscript.

Funding No funding is available.

Data availability Data are available from the authors on request (A.V.G.).

Code availability Not applicable.

Declarations

Ethics approval and consent to participate Not applicable.

Informed consent Not applicable.

Consent for publication Not applicable.

Research involving human participants and/or animals Not applicable.

Disclosure of potential conflicts of interest The authors declared no potential conflicts of interest concerning this article's research, authorship, and publication.

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