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Junction interaction in the seminiferous epithelium: regulatory roles of connexin-based gap junction

Nikki P. Y. Lee[1], William S. B. Yeung[2], and John M. C. Luk[1]

[1]Department of Surgery,and [2]Department of Obstetrics and Gynecology, Jockey Club Clinical Research Centre, The University of Hong Kong, Pokfulam, Hong Kong, SAR, China

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1. ABSTRACT

Anchoring junction, tight junction (TJ), and gap junction (GJ) constitute three major junction types in mammalian testes. Connexin is the well-studied GJ protein. It forms the building block of connexon, which is composed of six connexin units. Connexon forms the functional GJ when pairing with counter-connexon from neighboring cells. In the testis, at least eleven connexins are associated with the Sertoli and germ cells of the seminiferous epithelium and the Leydig cells of the interstitium, modulating spermatogenesis and steroidogenesis, respectively. Significantly, connexins are recently speculated to act as regulators of other junctions in the testes using pan-connexin peptide model. This demonstrates that the loss of connexin function leads to a preferential degradation of occludin-based TJ, but not N-cadherin-based adherens junction (AJ), in the testis, despite the intermingled relationship of these three junctions at the site of blood-testis barrier. In the clinical aspects, connexins are shown to relate to male infertility and testicular dysfunctions. A panel of molecules and proteins and their associated protein kinases are actively participating in the regulation of connexin-mediated GJ and fine-tuning connexin-associated functions in the testis. Herein, we summarize the latest findings of connexins in the testis in the aspects of fertility, and testicular diseases, with emphasis on the unexplored roles of connexins in regulating other junction types. This can shed light on future studies in implicating the putative roles of connexins in the physiological functions of reproduction and the clinical aspects of male infertility. In addition, understanding the roles of connexins can advance the diagnosis and treatment of testicular dysfunction and infertility.

2. INTRODUCTION

Gap junction (GJ), anchoring junction, and tight junction (TJ) are three major junction types that mediate intercellular adhesions and/or communications among different cell types in the testis, such as Sertoli and germ cells in the seminiferous tubules and myoid cells and Leydig cells in the peritubular and interstitial compartments (1, 2) (Figure 1). The testis consists of the seminiferous tubules and the interstitium, where spermatogenesis and

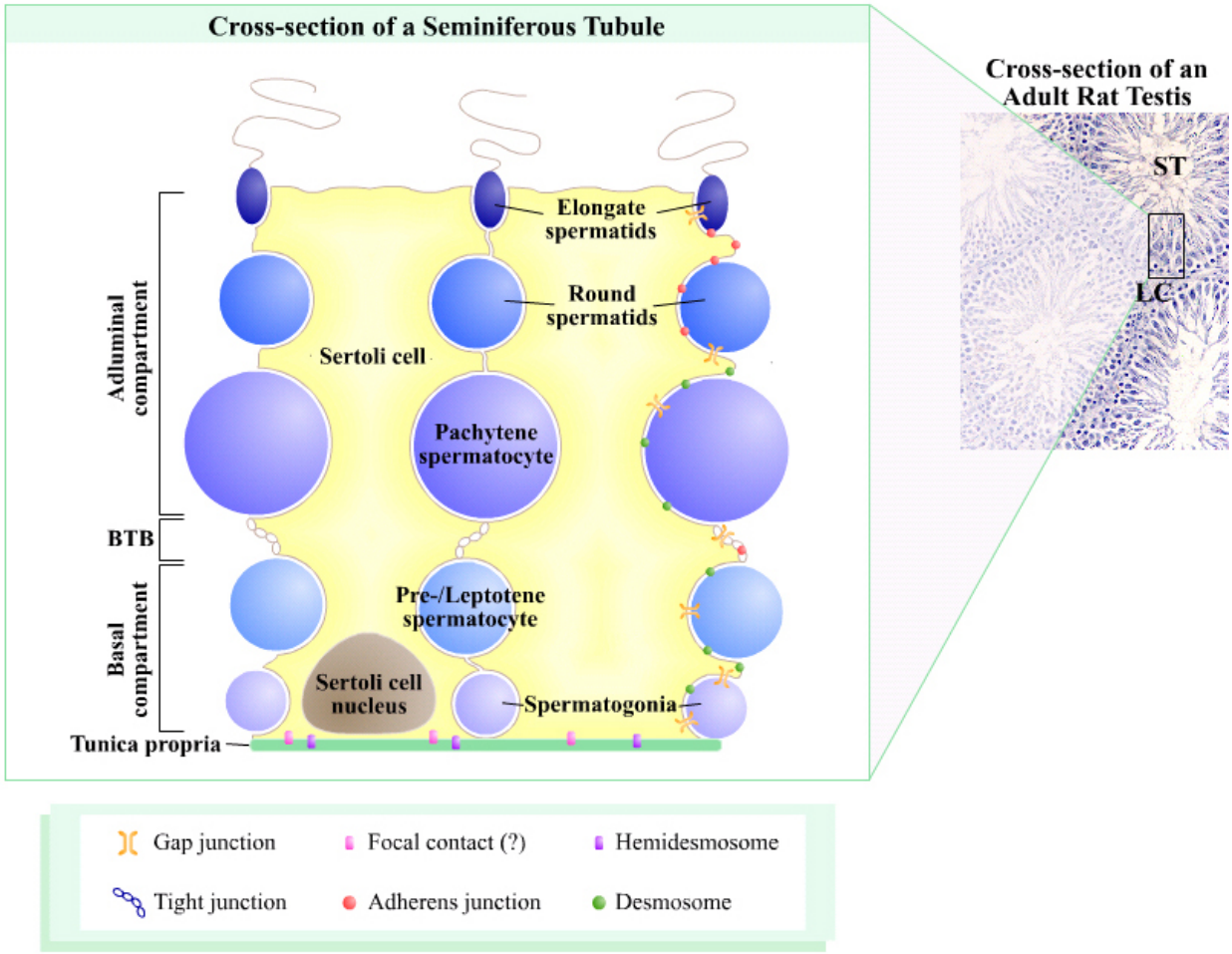

Figure 1. The intermingled relationship of AJ, TJ, and GJ in the testis. This is a schematic diagram presenting the localizations of junctions in the seminiferous tubules in the adult rat testis. Sertoli and various germ cells are found in the seminiferous tubules (ST), whereas Leydig cells (LC) situate in the inter-tubular compartments. Myoid cells are located in the peripheral area of the seminiferous tubules adjacent to tunica propria. During spermatogenesis, germ cells, such as spermatogonium, migrate from the basal compartment to the adluminal compartment, passing the blood-testis barrier (BTB), before releasing into lumens as mature spermatozoa. The movement of germ cells across the seminiferous epithelium involves rapid cell-cell associations and dissociations, which in turn activate restructuring of junctions, including GJ.

steroidogenesis occur, respectively (3). The blood-testis barrier (BTB) divides the seminiferous epithelium into the basal compartment and the adluminal compartment. During spermatogenesis, spermatogonia initially residing at the basal seminiferous epithelium migrate to the adluminal compartment in the course of development and undergo morphological transformation prior to releasing into the lumens as spermatozoa. The spermatogenic development of spermatogonia is primarily divided into several stages, such as pre- and leptotene spermatocytes, pachytene spermatocytes, round spermatids, and elongated spermatids (Figure 1). The continual generation of spermatozoa depends on the timely movement of pre- and leptotene spermatocytes across the BTB. These germ cells continue to develop and differentiate after passing the BTB. Along the path of germ cell migration and maturation, junction assembly and disengagement occur to facilitate the movement. Different junctional types in the BTB have been reviewed individually (2, 4, 5). Recent data suggest that these junctions are not functioning independently in the testis; in particular, the gap junctional molecules, connexins are putative modulators of other junctions. The present article reviews the role of connexins in testis with focus on the interaction of the junctional molecules.

3. ADHERENS JUNCTION AND TIGHT JUNCTION

Junctions in the testes are broadly classified into several types, according to their testicular locations and the underlying cytoskeletons attached (6). Among them, cadherin-based adherens junction (AJ) and occludin-based TJ are the two most well-studied junctions in the testis (6-8). In particular, testis possesses unique AJ types, named as ectoplasmic specializations and tubulobulbar complexes. The formers are found at the apical and basal seminiferous epithelium, whereas the latter is largely localized at the

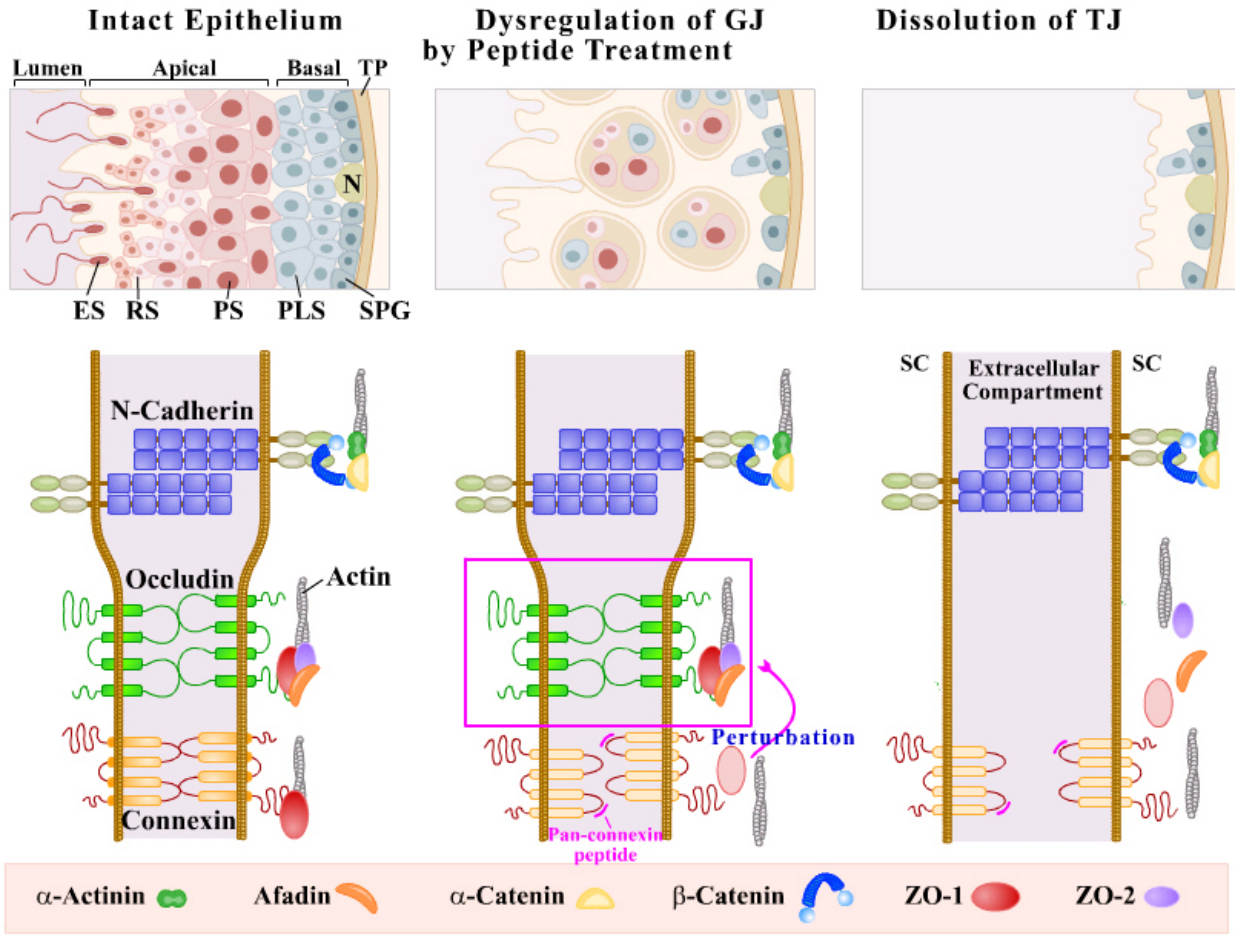

Figure 2. Morphology of the testes and junction structures of the blood-testis barrier (BTB) after treatment with pan-connexin peptide. Using pan-connexin peptide as a model, the interactive relationships of different junction types at the sites of BTB are demonstrated schematically (28). This is a speculative model based on the study using pan-connexin peptide, attempting to illustrate the BTB integrity after perturbation of the connexin structures in the testes (28). In normal rat testes, the intact epithelium contains several germ cell layers, composed of spermatogonia (SPG), preleptotene or leptotene spermatocytes (PLS), pachytene spermatocytes (PS), round spermatids (RS), and elongated spermatids (ES). BTB, physically dividing the seminiferous epithelium into adluminal and basal compartment, harbors hybrid junctions with AJ (N-cadherin-based), TJ (occludin-based), and GJ (connexin-based). After blocking GJ function by using pan-connexin peptide, multi-nucleated giant cells are generated within the seminiferous epithelium. The dysregulation of GJ concomitantly perturbs occludin-based TJ complex, leading to a subsequent elimination of occludin at the sites of BTB (indicated by the blunted arrow). At this time, most of the germ cells are lost, leaving behind the Sertoli cells, spermatogonia, and few spermatocytes in the seminiferous epithelium. However, no change in the level of N-cadherin is observed at the site of BTB. From this, it is known that occludin-based TJ is more susceptible to the depletion of connexin function in the testes. GJ, gap junction; N, Sertoli cell nucleus; SC, Sertoli cell; TJ, tight junction; TP, tunica propria; ZO, zonula occludens.

apical region (6, 9, 10). Different junctional components are identified in these junctions. For instance, the cadherin-based AJ complex comprises at least N-cadherin, β-catenin, and α-catenin, while the occludin-based TJ complex consists of occludin, zonula occludens (ZO)-1, and ZO-2 (Figure 2). In common, these two junction complexes utilize actin as their underlying cytoskeleton (Figure 2) (6, 11). Additionally, these adaptors are postulated to mediate junction cross-talks by means of the dynamic changes of the adaptors at these sites (12). Notably, AJ and TJ were previously found to interact with each other in the testes via several underlying adaptors (13).

4. GAP JUNCTION

GJ is composed of at least three families, namely connexins (14, 15), pannexins (16), and innexins (17). The connexins and pannexins are found in vertebrates with distinct tissue distribution patterns (14, 16), whereas the innexins are restricted in invertebrates (17). Pannexin is recently discovered, having three members, namely pannexin 1, 2, and 3 (16). Pannexin 1 and 2 are predominantly expressed in the central nervous system, but can also be found in eye, thyroid, prostate, kidney, and liver, whereas pannxin 3 is detected in skin only (16). By

contrast, connexin has more than 20 members and exhibits wide tissue distribution patterns (14, 15).

Until recently, seminiferous tubules are shown to express at least eleven connexins, including connexin (Cx) 26, 31, 31.1, 32, 33, 37, 40, 43, 45, 46, and 50 by semi-quantitative RT-PCR (5, 18). These connexins exhibit distinct and overlapping localizations within the seminiferous tubules (5). For instance, Cx 31 is found predominantly in the germ cells, whereas Cx 43 is found mainly in Sertoli cells (19, 20). Interestingly, most of the Cx 43 migrates from the apical to the basal compartments of the seminiferous epithelium during postnatal testicular development (21, 22). In particular, Cx 43 is a spermatogenic stage-specific protein highly expressed in stage VII tubules (20, 21). These results demonstrate differential expression and stage-specificity of connexins in the testis. While these histological and morphological observations implicate the putative roles associated with connexin expression, no further examination was done on the functional roles of connexins in the aspect of junction regulation, germ cell development, and spermatogenesis. To overcome these deficiencies, connexin null mutant and human disease models are used to correlate connexins and spermatogenesis (23, 24) (see below). A functional study utilizing fluorescent dye loading demonstrated the presence of asymmetric coupling in different testicular cell populations, such as Sertoli and germ cells, in the testis (25), which further strengthens the presence of gap junctions between various testicular cell types (more studies are tabulated in Table 3). Besides, the extent of couplings between them varied; it was weak between Sertoli cells and germ cells at later stage of spermatogenesis, when compared to those between Sertoli cells and spermatogonia (25). Despite these observations, the definitive role of connexins in spermatogenesis needs further investigation.

Structurally, connexin has four transmembrane domains, two extracellular loops, one intracellular loop, one intracellular N- and C-terminus and is characterized by having three conserved and regularly spaced cysteine residues, which control proper foldings of the extracellular loops and both are located in extracellular loop 1 and 2 in all the connexin family members (14, 15). Six connexins are assembled to form a connexon with an aqueous pore, which acts as a channel to transport and exchange ions and small molecules of less than 1 kDa between adjacent cells, and two connexons from opposite cells interact with each other to form a functional GJ (14, 15). In addition, connexons have non-junctional role by acting as transporters between cell and extracellular environment and between different intracellular compartments (26, 27), and may possess anti-apoptotic property in testis (28). It is known that connexons transport oxidized nicotinamide-adenine dinucleotide (NAD^+) and adenine triphosphates (ATP) from cytosol in astrocytes to their surroundings (27). Apart from binding with its own molecular species, recent studies further demonstrated the structural interaction of connexins with other adaptors (e.g. ZO-1) and cytoskeletal elements (e.g. tubulins) (29, 30). The presence of these components enables their participations in mediating communications among different protein complexes. Collectively, these findings clearly illustrate the functional diversity of connexins.

5. CONNEXINS AND REGULATION OF OTHER JUNCTIONS

5.1. Coexistence of junctional types at BTB

GJ was found to be localized in the basal and adluminal compartments in the seminiferous epithelium of the rat testes using freeze fracture and thin section techniques (31). It is present between Sertoli-Sertoli cells and Sertoli-germ cells in various stages of development (32, 33). Supporting these histological observations, subsequent study demonstrated the presence of intercellular electrical couplings in seminiferous tubules (34). These intercellular coupling processes are regulated partly by secondary messengers, such as cAMP (35). Despite such observations, it is still unknown about the nature of these molecules involved and how they participate in these processes. Differed from other epithelia with distinct junction complexes, testes contain hybrid junctions that are distributed throughout the seminiferous epithelium (Figure 1). Notably, the N-cadherin-based AJ, occludin-based TJ, and connexin-based GJ are identified at the BTB site (2, 7, 12). These junctions intermingle with each other while they perform distinct functions. Specifically, BTB physically segregates the seminiferous epithelium into the basal and apical compartments (11). Such that, the translocation of preleptotene and/or leptotene spermatocytes from the basal to the apical compartment, germ cells need to pass through the BTB, which inevitably needs the restructuring of the BTB (7, 11). It is obvious that the coordination of different junction types is destined for securing the process and to substantiate spermatogenesis.

5.2. Structural basis of junctional interactions

It was previously postulated that adaptors were core proteins that mediated the interactions between different junction complexes (12). A recent study also demonstrated that adaptors were linkers for N-cadherin-based AJ and occludin-based TJ at the BTB site in the testes (13). In addition to the linker function, adaptors are also participated in cytokine signaling in the testis (8). Apart from adaptors, the underlying cytoskeletons, such as actin filaments, also play a role in bridging different protein complexes. Actin is shown to be the underlying cytoskeleton of both AJ and TJ in the testes (1, 12). As such, it is reasonable to understand the cross-talk of different protein complexes via the associated adaptors and underlying cytoskeletons.

5.3. Connexins and inter-junction cross-talks in the testes

GJ is widely distributed in the seminiferous epithelium, particular in the apical and basal compartments (31-33), and intertwined with TJ and AJ molecules in the testis (1, 36, 37) (Figure 1). Available data have suggested that connexins are putative modulators of other junctions, such as TJ, in the testis. The first evidence supporting this notion came from an *in vitro* study using Sertoli cell

cultures, which demonstrated the functional correlation between Cx 33 and an AJ protein, N-cadherin (38). When Sertoli cells were cultured to allow the formation of TJ and AJ, a transient induction of Cx 33 coincided with the induction in N-cadherin expression (38), suggesting a coordination of Cx 33 and N-cadherin expression during the TJ and AJ formation *in vitro*. Also supporting the existence of interaction between GJ and AJ is the induction of other AJ-associated proteins, such as β-catenin, similar to the trends observed in the Cx 33 during Sertoli cell junction formation *in vitro* (38, 39). Moreover, a significant correlation was found between the reduction in the protein level and cytoplasmic relocalization of Cx 43 with the decreased expression of N-cadherin, ZO-1, and/or TJ protein, occludin, when Sertoli cells were treated with a panel of reproductive toxicants, such as dieldrin (DED) and *tert*-octylphenol (OP) (40).

5.4. Pan-connexin peptide model as an alternated approach used in the study of junctional interactions in the testes

Efforts have been made to study connexins in the testis using GJ blockers, inhibitors, and reproductive toxicants, such as lindane (41) and cadmium chloride (40). The major drawback with these approaches is that such blocking/inhibitory reagents have broad spectrum of actions and thus hamper virtually all types of GJ and other junctions in the testis. For instance, cadmium chloride perturbs both AJ and TJ functions in testes (42). As a result, any observed effects of these reagents might be attributed to the secondary or side effects instead of the direct inhibitory effects on connexin-based GJ. In order to minimize the non-specificity of these reagents, we have used connexin-specific peptide to explore the essentiality of connexins. An *in vivo* model is established recently to investigate the functions of connexins by using connexin-specific peptides with amino acid sequences corresponding to the extracellular loop region of connexins (28). In this model, pan-connexin peptide is designed based on the conserved region in the first extracellular loop of all known connexins in the testes (28). This peptide works by mimicking the binding sites of opposite connexins, hampering the formation of functional GJ (28). The effects of connexin peptides in blocking the functional GJ are illustrated in several other studies (43, 44). On the other hand, the peptide specificity is further enhanced by designing peptides targeting to the extracellular loop regions that are highly specific for a particular connexin, such that blocking the function of one type of connexin will not affect the other type of connexin (45). In aortic smooth muscle cells expressing Cx 40 and Cx 43, Cx 43-specific peptide inhibited Cx 43 channel activity without disturbing the activity of Cx 40 channels (45). In our laboratory, Cx 31-specific and Cx 33-specific peptides, with amino acid sequences based on their distinct regions on the second extracellular loop of connexin, were used to selectively block the functionalities of Cx 31 and Cx 33 in the testes (28). However, no significant difference was observed when using these two peptides (28). This might be largely due to the compensatory effects of different connexins in the testes, similar to the cytokine network in the immune system.

Using this pan-connexin peptide model to block the functions of connexins in the testis, selective loss of occludin expression after germ cell depletion from the seminiferous epithelium was observed (28). However, the level of N-cadherin at the basal seminiferous epithelium remained relatively unaffected after the germ cell loss (28). This postulates that connexin is an immediate and preferential regulator of occludin-based TJ instead of N-cadherin-based AJ at the sites of BTB in the testes (Figure 2). The interaction among these junction proteins is feasible since these junction types share common adaptors, such as ZO-1, which can be found in AJ, TJ, and GJ (1, 12, 14, 46), such that the release or incorporation of adaptors from one junction type might interfere with the junction complexity and stability of other junctions (12). In addition, these junctions utilize common underlying cytoskeletons, such that disruption of one junction can directly affect the structural stability of other junctions (12). For instance, Cx 43 is associated with tubulin-based microtubules, which are also used by cadherin-based protein complexes (47). As such, it is feasible that different types of junctions can communicate and interact with each other via these extensive networks.

5.5. Connexins and junction interactions in other systems as implications for future study in the testes

Junction interactions involving connexins as participants are not restricted merely in testes. Connexins have demonstrated versatile interactions with other junction members in other epithelia or endothelia in several other studies. The interrelated nature of AJ and GJ was demonstrated by blocking GJ-mediated dye transfer in Novikoff cells using antibodies directed against N-cadherin to disrupt N-cadherin-based AJ (48). Besides, transfection of immortalized mouse hepatic cells with Cx 32 associated with the induction of a panel of TJ integral proteins and adaptors, namely occludin, claudin-1, and ZO-1 (49), strengthening the TJ functionality. Subsequent cDNA microarray analysis further postulated that this interaction was in part mediated by the up-regulation of membrane-associated guanylate kinase with inverted orientation-1 (MAGI-1), which is a common adaptor found in TJ and AJ (50). On the other hand, upregulation of claudin-1 was accompanied with down regulation of Cx 43 and occludin, a TJ protein, in interleukin-1β-treated primary human astrocytes (51), suggesting a reciprocal relationship between GJ and TJ. However, another study using *in vitro* testicular system reported that dislocalization of Cx 43 in lindane (GJ blocker)-treated Sertoli cells did not alter the localization of occludin (41). These results implicate the diversity of connexins in regulating TJ in different systems. In spite of the limited functional data of connexins available in the testes, it is still speculated that connexins also associate with other junctions similar to those exhibited in other systems.

5.6. Interaction among connexin molecules

On the other hand, it is suggested that connexins regulate the expressions of other connexin members. Transfection experiments demonstrated that Cx 33 hindered the channel formation mediated by Cx 37 and 43, but not Cx 32 (52). Interestingly, the extent of inhibitions varied

Table 1. Connexin mutants

Connexins	Viability	Defects leading to lethality	Fertility	References[1]
Singly mutant mice				
Cx $26^{-/-}$	Embryonic lethality dpc 11	Starvation	n.k.	73
Cx $31^{-/-}$	Viable[2]	N.A.	Fertile	74
Cx $32^{-/-}$	Viable	N.A.	Fertile	55
Cx $37^{-/-}$	Viable	N.A.	Sterile F; Fertile M	54
Cx $40^{-/-}$	Viable	N.A.	Fertile	75, 76
Cx $43^{-/-}$	Neonatal lethality	Heart	Sterile	77, 78
Cx $45^{-/-}$	Embryonic lethality dpc 10	Heart	n.k.	79
Cx $46^{-/-}$	Viable	N.A.	Fertile	80
Cx $47^{-/-}$	Viable	N.A.	Fertile	56
Cx $50^{-/-}$	Viable	N.A.	Fertile	81
Doubly mutant mice				
Cx $32^{-/-}43^{-/-}$	Neonatal lethality	Heart	n.k.	82
Cx $32^{-/-}47^{-/-}$	Lethality dpp51	Myelination	n.k.	56
Cx $37^{-/-}40^{-/-}$	Perinatal lethality dpp1	Vascular	n.k.	83

[1], References listed herein are selected references only. While every effort was made to cite the original articles, it is noted that some important works would have been missed in this table. [2] ~60 % of connexin $31^{-/-}$ mice were lost between dpc 10.5 and 13.5 (74) Abbreviations: dpp, postnatal day; dpc, post coitum; Cx, connexin; F, female; M, male; N.A., not applicable; n.k., not known

Table 2. Connexin-related testicular abnormalities

Connexin-related abnormalities	Observations	References[1]
Leydig cell tumorigenesis	Sequestration of Cx 43 in early endosome	62
Altered spermatogenesis	Diminution of the level of Cx 43	20
Spermatogonial arrest	Abnormal localization and expression level of Cx 43 and 26	61
Azoospermia	Diminution of the level of Cx 43 in the tubules	24
Germ cell deficiency	Null mutation of Cx 43	23, 78

[1], References listed herein are selected references only. While every effort was made to cite the original articles, it is noted that some important works would have been missed in this table.

between different connexins, such that Cx 33 had a stronger inhibitory effect against Cx 37 when compared to Cx 33, suggesting that the inhibition was connexin-specific (52). In particular, different rates of disappearance of connexin family members, such as Cx 31, Cx 32, and Cx 43, were observed after pan-connexin peptide treatment, strengthening the diversity of connexin interactions in the testes (28).

6. CONNEXINS AND FERTILITY

With the exception of some connexin mutants, most of the connexin mutants are viable and fertile, diminishing the significance of connexins in modulating viability and fertility (53) (Table 1). This is not surprising in view of the presence of more than eleven connexin members in the testis and new members are being discovered (18); these connexins complement the roles of other connexin when it fails to perform its functions. Interestingly, Cx 37 mutants demonstrate dual roles in controlling fertility in male and female mice, resulting in sterile female and fertile male (54). In order to further study the roles of connexins in viability and fertility, double connexin mutants with distinct characteristics from their corresponding single mutants, were generated (Table 1).

For instance, single Cx 32 mutants and single Cx 47 mutants are viable and fertile (55, 56), whereas double Cx 32 and Cx 47 mutants are lethal (56). These observations further clarify the complementary roles of different connexins. Besides, several connexins, such as Cx 31, Cx 33 and Cx 30.2, were found to be predominantly (38, 57) or specifically expressed (58) in the testis, implicating that these connexins have essential roles in the testis. In short, connexins have significant roles in controlling viability and fertility in mice.

7. CONNEXINS AND TESTICULAR DISEASES

Connexins are known to be one of the central regulators of cancers in different organs and epithelia (59). Several testicular models, such as Sertoli cell-only (SCO) syndrome, treatment of Sertoli cells with lindane, and infiltration of testis with carcinoma-*in-situ* (CIS) or seminoma, were developed and utilized to investigate the role of connexins in overcoming tumorigenesis and aspermatogensis (20, 24, 41, 60, 61) (Table 2). Significant reduction in the protein level and dislocalization of Cx 43 were observed during aspermatogenesis and tumorigenesis in seminoma cells (24, 60, 61). In *ebo/ebo* and *jun-d*$^{-/-}$ mutants with impaired spermatogenesis, there was a reduction in the level of Cx 43 (20). Furthermore, the level of cytoplasmic Cx 26 was induced in Sertoli cells in the seminiferous tubules infiltrated with CIS or seminoma, whereas the level of Cx 43 was declined accordingly (61). These observations are associated with altered connexin levels and aberrant connexin localizations to testicular dysfunction. On the other hand, aberrant cytoplasmic Cx 43 was shown to localize within early endosomes in tumor Leydig cells (62) and Golgi apparatus in human seminoma cells (60), implicating that connexins was also involved in other regulatory or transport pathways in tumor-induced dysregulation. Significantly, transfection of Cx 43 in human testicular seminoma cells restored the membrane localization of connexin and functional cell coupling between cells, and reversed the rapid proliferation of the seminoma cells (60). Apart from Cx 43, the effect(s) of other connexins in promoting or reversing tumorigenesis await further studies.

Table 3. Hormonal and signaling regulators of connexins

Regulators	Targeted connexins	Effects[1]	Cell types/Organs Involved	Techniques	References[2]
cAMP	Cx 43	-	Leydig cell	Dye Transfer	65
	unknown	-	Seminiferous tubules, Sertoli cell	Electrotonic Coupling	35
	unknown	+	Sertoli cell	Electrical and Dye Couplings	64
LH	unknown	-	Leydig cell	Dye Transfer	65
PKA	unknown	-	Leydig cell	Dye Transfer	65
PKC	unknown	-	Leydig cell	Dye Transfer	65
Retinoid	Cx 43	+	Seminiferous tubules	Dye Coupling	21
T	unknown	-	Leydig cell	Dye Transfer	65

[1], +, Positive effect in GJ coupling; -, negative effect in GJ coupling, [2], References listed herein are selected references only. While every effort was made to cite the original articles, it is noted that some important works would have been missed in this table. Abbreviations: cAMP, cyclic AMP; Cx, connexin; LH, luteinizing hormone; PKA, protein kinase A, PKC, protein kinase C; T, testosterone

8. REGULATION OF CONNEXINS IN THE TESTIS

The distinct and overlapping roles of different connexin members in spermatogenesis raise questions on how connexins are regulated and coordinated to achieve this high level of complexity and functionality. Similar to other junctions (1, 6, 7), GJ is modulated by similar factors and regulators, such as hormones and ions (Table 3). If the underlying mechanisms of these regulators are fully delineated, then they can be used for future studies to fine-tune the levels and subsequently the functions of connexins. In addition, the regulatory mechanisms can become prime targets to modulate the expressions and levels of connexins to reverse testicular tumorigenesis, which is partly contributed by the dysregulation of connexins (see above). Following is description of certain endocrine factors and signaling modulators that regulate the levels and functions of connexins and GJ in the testis (Table 3).

8.1. Hormones

Spermatogenesis is a process largely regulated by hormones, such as luteinizing hormone (LH) and testosterone (T) (1, 3, 63). As such, it is speculated that connexins are also subjected to hormonal regulation in the testis. Previous study had shown that Sertoli cell dye couplings were induced in the presence of follicle-stimulating hormone (FSH), when assessed by microinjection of Lucifer Yellow fluorescent dye (64). On the contrary, LH-induced T caused uncoupling of Leydig cells in vitro (65). In addition, 17β-estradiol and T, when added intracellularly and extracellularly, impaired Sertoli cell communications in a dose-dependent manner (66). In particular, esterified forms of T and 17β-estradiol have stronger uncoupling effects than the nonesterified forms, probably due to the liposoluble nature of the ester chains (66). Despite that, it is still not fully understood the regulatory mechanisms of hormones on the regulation of connexins. Based on studies performed in other models, protein kinase A (PKA) and mitogen-activated protein kinase (MAPK) are putative downstream targets. For instance, LH inhibited the translation of Cx 43 in ovarian follicles via PKA/MAPK-dependent pathway (67). In brief, these observations suggest that hormones exert divergent effects on the levels and activities of connexins.

8.2. Secondary messengers and associated protein kinases

The roles of secondary messengers, such as cAMP and cGMP, and their associated protein kinases, namely PKA and protein kinase G (PKG), in the regulation of the integrity of TJ and AJ in the testis have been well characterized (6, 7). In fact, cAMP and PKA have been shown to regulate GJ in the testis as well. Earlier study utilizing electrophysiological techniques demonstrated that the cell-cell couplings in Sertoli cells and seminiferous tubules were inhibited by a modified form of cAMP, dibutyryl cAMP, (35), which reduced the number of coupled Leydig cells *in vitro* (65). Specifically, in the uncoupling condition-mediated by dibutyryl cAMP treatment, which was reversed by staurosporin (a protein kinase inhibitor), Cx 43 demonstrated a diffuse cytoplasmic localization in Leydig cells (65). On the contrary, cAMP favored GJ communications in Sertoli cells (64). In fact, cAMP also acts as a regulator of GJ communications in other epithelia. For instance, cAMP reversed the uncoupling effects of dimethylnitrosamine, a renal carcinogen, in renal epithelial cells (68). Evidences are still lacking to delineate the underlying regulatory pathways of these secondary messengers, but it is likely that PKA and PKG are two of the downstream proteins that are involved. As it is known that associated protein kinases of these secondary messengers and other protein kinases and phosphatases, such as MAPK and protein tyrosine phosphatase, also participate in the GJ regulation (69, 70).

8.3. Retinoids

Retinoids, derivatives of vitamin A, are positive regulators of GJ in the testis. In retinoid X receptor β-deficient mice, the expression of Cx 43 was greatly reduced in the seminiferous epithelium when assessed by *in situ* hybridization (21), suggesting that retinoids are one of the important regulators of Cx 43 expression. This observation was further supported in another model, the vitamin A-deficient (VAD) post vitamin A administration (PVA) model. The occurrence of spermatogenesis arrest in the VAD rats confirms the importance of vitamin A in maintaining spermatogenesis (58, 71). Using the VAD-PVA rat models, we found differential expression of Cx 31 and Cx 43 in rat testes with different stages of spermatogenesis (58). For instance, mRNA of Cx 43 could not be detected in the VAD rat testis with arrested spermatogenesis. Administration of vitamin A to these animals re-initiate the expression of Cx 43 in day 15 VAD-PVA rat testes (58) . The mRNA level of Cx 43 was elevated and remained high thereafter (58). Similarly, retinoid X receptor β-deficient mice are sterile (72). These results suggest that vitamin A and its derivatives, retinoid,

are important regulators to modulate GJ functioning and spermatogenesis in the testis. One possible mechanism leading to aspermatogenesis may involve the blockage of the vitamin A/retinoid-mediated pathway.

9. CONCLUSIONS AND FUTURE PERSPECTIVES

GJ is one of the major junction types being studied, in addition to TJ and AJ, in the testis. Although three GJ proteins, namely connexins, pannexins, and annexins, are found today, only connexins are positively identified in the testis. More than 11 connexins with varied sizes and testicular localizations have been reported to express in the testis. Furthermore, connexins can mediate homomeric/heteromeric and/or homotypic/heterotypic interactions. Such diversities as demonstrated by the connexins could amplify their roles in spermatogenesis and steroidogenesis. Pan-connexin peptide blockage model is developed as an alternative to investigate the functional significance of connexins in the testes. By this approach, connexins are postulated to selectively regulate occludin-based TJ, but not N-cadherin-based AJ. In this aspect, it is still unknown what types of signaling proteins or adaptors/underlying cytoskeletons are involved in connexin-mediated TJ regulation in the testes. In addition, connexins are demonstrated to possess anti-apoptotic properties in the testes. To address these issues, the downstream signaling proteins of connexins and their respective inhibitors deem further investigation. Although, most of the connexin deficient mice are viable and fertile, dysfunctions of connexins may represent one of the triggers causing testicular tumorigenesis and aspermatogenesis. Due to the essentiality of connexins in maintaining the normal function of testes, it is not surprising to know that connexins are subjected to regulation by an array of molecules and proteins, such as hormones. In spite of the current knowledge about connexins in the testis, questions are still waiting to be answered. For instance, will the ratios and combinations of different connexins contribute to the stability and functionality of GJ in the testis? And how these parameters affect aspermatogenesis? Also, is it possible to reverse testicular tumorigenesis if the levels of deficient connexins are restored? Importantly, whether connexin-based treatment is a putative medical cure for testicular tumorigenesis? Before these concerns are addressed, it is important to carry out in-depth studies to elucidate the unique functions associated with each connexin. Results concluded from these studies can advance future studies about the diagnosis and treatment of male reproductive dysfunction.

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Send correspondence to : Dr. John M. Luk, Department of Surgery, The University of Hong Kong, Hong Kong, SAR, China. Fax: 852-28199636, E-mail: jmluk@hkucc.hku.hk; Prof. William Yeung, Department of Obstetrics and Gynecology, The University of Hong Kong, Hong Kong, China. Tel: 852-28553405, E-mail: wsbyeung@hkucc.hku.hk