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Review

Gap junctional communication in the male reproductive system

Georges Pointis [a,*], Céline Fiorini [a], Norah Defamie [a,1], Dominique Segretain [b]

[a] *INSERM U 670, Faculté de Médecine, 28 avenue de Valombrose, 06107 Nice cedex 02, France*
[b] *Université Paris V, 45 rue des Saint-Pères, 75006 Paris, France*

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Abstract

Male fertility is a highly controlled process that allows proliferation, meiosis and differentiation of male germ cells in the testis, final maturation in the epididymis and also requires functional male accessory glands: seminal vesicles, prostate and corpus cavernosum. In addition to classical endocrine and paracrine controls, mainly by gonadotropins LH and FSH and steroids, there is now strong evidence that all these processes are dependent upon the presence of homocellular or heterocellular junctions, including gap junctions and their specific connexins (Cxs), between the different cell types that structure the male reproductive tract. The present review is focused on the identification of Cxs, their distribution in the testis and in different structures of the male genital tract (epididymis, seminal vesicle, prostate, corpus cavernosum), their crucial role in the control of spermatogenesis and their implication in the function of the male accessory glands, including functional smooth muscle tone. Their potential dysfunctions in some testis (spermatogenic arrest, seminoma) and prostate (benign hyperplasia, adenocarcinoma) diseases and in the physiopathology of the human erectile function are also discussed.

Keywords: Gap junction; Connexin; Male reproductive tract; Fertility; Pathogenesis

Contents

* Corresponding author. Fax: +33 4 93 37 77 31.
E-mail address: pointis@unice.fr (G. Pointis).
[1] Present address: Equipe ICC, IPBC, UMR CNRS 6187, Pôle Biologie Santé, Université de Poitiers, 40 avenue du recteur pineau, 86022 Poitiers cedex, France.

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1. Introduction

Testis exerts two major functions, an endocrine function characterized by synthesis of androgens, and an exocrine function via spermatogenesis. Spermatogenesis, which takes place within seminiferous tubules, is a highly controlled process that may be divided into three principal phases: spermatogonia renewal and proliferation, spermatocyte meiosis and spermatid differentiation [1]. In addition, developing germ cells must progressively migrate from the basal to the luminal compartment of seminiferous tubules so that fully differentiated spermatids can be released into the lumen at spermiation. Within the seminiferous epithelium, spermatogenesis is supported by Sertoli cells that provide structural and nutritional supports for the developing germinal cells (reviewed in Ref. [2]).

Spermatogenesis requires a functional hypothalamo-pituitary system involving Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) and a local control via paracrine signals. FSH regulates spermatogenesis via stimulating the proliferation of Sertoli cells during the neonatal period. LH indirectly controls spermatogenesis through androgens produced by Leydig cells located in the interstitial compartment between seminiferous tubules. Androgens play also a primordial role in the initiation and maintenance of the male reproductive tract. Testosterone is the predominant circulating androgen and is necessary for the differentiation of the Wolffian ducts into the seminal vesicles, epididymis, and ejaculatory ducts. The 5-α reduced product of testosterone, dihydrotestosterone, is needed for the differentiation of the urogenital sinus and genital tubercle into the prostate, urethra, penis, and scrotum (reviewed in Ref. [3]).

Local cell–cell interactions including direct contact-dependent junctional pathways are also essential in the regulation of mammalian spermatogenesis and in the maintenance of the male phenotype [4,5]. These cellular junctions are composed of specialized proteins implicated in adhesion (such as cadherin–catenin, nectin, integrin and others) and attachment (such as occludin, claudins, Zonula occludens and others) (reviewed in Ref. [6]). A third type of junctions present in the testis and in the male genital tract (epididymis, seminal vesicle, prostate, corpus cavernosum) concerns gap junctions and their constitutive proteins, connexins (Cxs).

The present review has been designed to update the recent developments on Cx identification in the male reproductive tract and their role on male fertility.

2. Gap junction channels and connexins

Gap junctions are clusters of plasma membrane channels formed of Cxs, which allow direct intercellular exchange of molecules of molecular weight smaller than 1 kDa (ions and signaling mediators) between the cytoplasmic compartments of two adjacent cells (reviewed in Ref. [7]). Today, at least 20 Cxs differentiated by their molecular weights are identified (reviewed in Ref. [8]). They are expressed in a tissue-specific manner and various cell types exhibit more than one Cx. Cxs oligomerize to form two juxtaposed hemichannels termed connexons, which are defined as homomeric when composed of the same Cx or heteromeric when at least two different Cxs form the connexon. Gap junctional intercellular communication (GJIC) is involved in the regulation of cell growth and differentiation, homeostasis and neoplastic transformation (reviewed in Ref. [9]). Recent studies have reported that Cxs, by themselves, can play critical functions in signal transduction, independent of forming intercellular channels (reviewed in Ref. [10]).

3. Gap junction and connexin expression in the testis

Testicular gap junctions were first described by electron microscopy in the rat testis [11]. By using freeze fracture electron microscopy and analysis of the junctional permeability with fluorescent dyes, the presence of these junctions was further reported in the testis of rodents prior to and during the initiation of spermatogenesis [4,12–14].

In the mature rat testis, mRNA for eleven different Cxs (e.g., Cx26, Cx30.2, Cx31, Cx31.1, Cx32, Cx33, Cx37, Cx40, Cx43, Cx46 and Cx50) have been detected [15–20]. Ten of the Cx mRNAs examined were present in polysomes and presumably translated in adult seminiferous tubules [19]. Transcripts for Cx26, Cx30.3, Cx31, Cx31.1, Cx32, Cx37, Cx40, Cx43, Cx45 and Cx46 were also found in the fetal mouse testis [21].

3.1. Gap junction and connexin expression within the seminiferous epithelium

Within the seminiferous tubules, gap junctions are located in the region of Sertoli cell occluding junctions at the level of the blood–testis barrier [22,23] and between Sertoli and germ cells [24,25]. The distribution of Cx mRNA and protein in the different cell types of the testis is summarized in Table 1.

Cx43, the predominant Cx in the testis, is located in the basal compartment of the seminiferous tubules in rat [26–28], guinea pig, mink [29] and human [30,31]. The specific distribution of Cx43 within the seminiferous epithelium was evidenced by in situ hybridization experiments demonstrating that Cx43 mRNA was expressed by Sertoli cells and basally located germ cells such as spermatogonia and spermatocytes in testes of rat [32] and human [31,33]. Deconvolution microscopy analysis, which allows a better resolution that confocal analysis, clearly indicated that Cx43 was mainly present at the

Table 1
Localization of Cx mRNA and proteins in the testis

Cx	Species	Testicular cell types in which Cx mRNA and proteins are detected	References
Cx26	mouse, rat	Spermatocytes, spermatids (RT-PCR)	[19]
		Apical compartment of seminiferous tubules (IHC)	[26]
	human	Seminiferous tubules (cell type not identified)	[49]
Cx30.2	mouse	Leydig cells and endothelial cells (IHC)	[20]
Cx31	rat	Spermatocytes, spermatids and peritubular cells (RT-PCR)	[19]
		Spermatogonia, spermatocytes (ISH, IHC)	[47]
Cx31.1	rat	mRNA detected in the testis but unknown localization	[19]
Cx31.9	human	Vascular smooth muscle	[20,50]
Cx32	rat, mouse	Spermatocytes, spermatids and Sertoli cells (RT-PCR)	[19]
		Apical compartment of seminiferous tubules (IHC)	[26]
	fish	Sertoli cells, spermatogonia and spermatocytes (WB; IHC)	[40]
Cx33	rat, mouse	Sertoli cell (WB, IHC)	[41,43,44]
		Spermatocytes and spermatids (RT-PCR)	[19,45]
Cx37	rat	Peritubular cells (RT-PCR)	[19]
		Endothelial cells (IHC)	[41]
Cx40	rat	Spermatocytes, spermatids and peritubular cells (RT-PCR)	[19]
	human	Testicular cell types not identified (RT-PCR)	[51]
Cx43	rat, mouse	Sertoli cells, Leydig cells, peritubular cells (IHC, IG, RT-PCR)	[26,28,29,32,43,52,55,60,65,76,110]
	Guinea pig, mink	Sertoli cells, Leydig cells (IHC)	[29]
	human	Sertoli cells, Leydig cells, spermatogonia (ISH, IHC)	[30,31,49]
	frog	Sertoli cells, Leydig cells, spermatogonia (IHC)	[52]
Cx45	rat	Peritubular cells (RT-PCR)	[19]
Cx50	rat	Spermatocytes, spermatids (RT-PCR)	[19]

base of Sertoli cells (identified by vimentin immunostaining, a Sertoli cell specific marker) in close association with adjacent Sertoli cells and with vimentin-negative cells that are likely germ cells (Fig. 1). At this compartmental level, Cx43 colocalized with occludin, a tight junction protein [34] and with the associated tight junction protein, Zonula Occludens-1 (ZO-1) [28]. However, a more discriminating analysis of Cx43 and ZO-1 signals reveals that, in all cases examined, a close colocalization was often unusual [35]. Such an absence of complete association between Cx43 and ZO-1 was also reported in myocytes [36].

Dye coupling experiments performed in situ, with a large variety of low-molecular-weight tracers, reveal that there are multiple routes of GJIC in rat seminiferous tubules that differ in permeability properties and show alternative gating states [37]. By developing a sophisticated in situ assay that allows correlating dye transfer, cell type identification and Cx43 localization, we showed that Cx43 participates in the coupling between adjacent Sertoli cells, between Sertoli cells and spermatogonia, between Sertoli cells and early and late spermatocytes but not between Sertoli cells and spermatids [38]. Our study also indicates that the coupling, analyzed with Lucifer yellow, appeared unidirectional from Sertoli cells to germ cells, whereas no transfer of this dye was observed from germ cells to the somatic cells. These data are in agreement with those reported by other authors but do not exclude that other low-molecular-weight tracers that differ in their physicochemical properties could diffuse from spermatogonia to Sertoli cells. According to Risley et al., this asymmetric coupling would facilitate the concentration in germ cells of

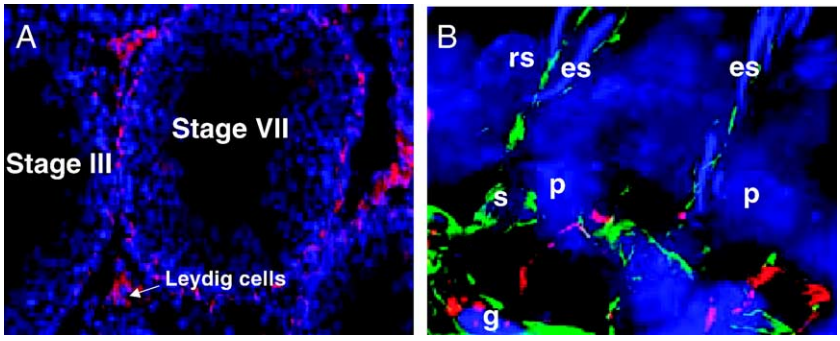

Fig. 1. Localization of Cx43 in a cross section of the adult rat testis. (A) The Cx43 immunosignal (red fluorescence) is located in the basal compartment of the seminiferous tubules and in the interstitial compartment between Leydig cells. Note that the Cx43 signal is dependent upon the spermatogenic stages. Nuclei of Leydig, germ and Sertoli cells were identified by DAPI staining (blue fluorescence) (×60). (B) High-resolution deconvolution microscopy analysis of the basal compartment of a rat seminiferous tubule at stage V of spermatogenesis (×600). The vimentin filaments (green fluorescence) underline the Sertoli cell cytoplasms. Cx43 staining (red fluorescence) is located at the base of the Sertoli cell and present close contact with vimentin filaments. Nuclei of early and late germ cells and of Sertoli cells were identified by DAPI staining (blue fluorescence). s: Sertoli cell; g: spermatogonia; p: pachytene spermatocyte; rs: round spermatid; es: elongated spermatid.

molecules diffusing through junctions from Sertoli cells [37]. From altogether these data, it has been suggested that gap junctions present between these two cell types may participate in the control exerted by Sertoli cells on germ cells.

A specific feature of spermatogenesis is that the dividing germ cells fail to complete cell division resulting in the formation of cytoplasmic bridges. These structures interconnect a large number of germ cells and allow synchronous development of these cells by sharing gene products [39]. The possibility that gap junctions in concert with cytoplasmic bridges participate in exchanges between germ cell cytoplasms must be considered. In fish, where spermatogenesis occurs in cysts rather than in seminiferous tubules, gap junctions were characterized between germ cells [40]. However, no gap junctions were described between germ cells in mammals.

The presence of Cx43 was also detected in residual bodies, the excess of germ cell cytoplasms which are phagocytosed by Sertoli cells during spermiation (unpublished data). Whether this Cx accumulation reflects a degradation mechanism of the protein or is related to another unknown role of Cx in residual bodies is presently not clear.

In contrast to Cx43 which was mainly located in the basal compartment of seminiferous epithelium, Cx26 and Cx32 were only found in the apical region of the rat seminiferous tubules but the cell types which express these Cxs were not identified [26]. Cx30.2, the putative mouse orthologue of human Cx31.9, was detected at high levels in vascular smooth muscle cells and at a low level between Sertoli and germ cells, and in Leydig cells [20].

In rat and mouse, Cx33 is mainly restricted to the testis [17,41] and to hippocampal multipotent progenitor cells [42]. Immunohistochemical studies demonstrated that Cx33 was present in the basal compartment of seminiferous tubules and not found in Leydig cells [41,43]. Its presence was clearly shown in Sertoli cells when immunohistochemistry analysis was performed on testis sections [44] and in vitro in a Sertoli cell line [43]. Other data reported that Cx33 was not only restricted to Sertoli cells but was also detected by RT-PCR in both crude preparation of germ cells [45] and in enriched preparations of mature male germ cells such as pachytene spermatocytes and round spermatids [19]. However, due to the difficulty to obtain pure fractions of germ cells, the presence of Cx33 transcripts in these cell preparations is doubtful. Another typical feature of Cx33 is that its expression is different from that of Cx43. While the intensity of the immunoreactive signal for Cx43 was markedly reduced in the abdominal testis of a cryptorchid mutant rat, Cx33 was maintained at a high level in Sertoli cells (Fig. 2, upper panels). As observed above for the protein, semiquantitative analysis of Cx33 and 43 transcripts reveals that the level of Cx33 mRNA was not altered in the cryptorchid testis while Cx43 was significantly reduced in the pathological testis (Fig. 2, lower panels). The reasons for these discrepancies have not yet been elucidated. Another interesting point is that Cx33, in contrast to other Cxs, has been reported to exert a Cx-specific inhibitory effect on GJIC when mRNAs were microinjected into paired *Xenopus* oocytes [46]. In Sertoli cells, our laboratory demonstrated that sequestration of a Cx33/Cx43 complex was associated with a complete inhibition of the junctional coupling between adjacent cells [43]. These findings provide the first evidence of a new mechanistic model by which a native Cx, exerting a dominant negative effect, can inhibit GJIC.

Within the testis, other Cxs have been only detected in germ cells. Cx31 mRNA was specifically localized by in situ hybridization in spermatogonia and early spermatocytes (leptotene and zygotene) and its presence was confirmed by the detection of an immunoreactive signal in the cytoplasms of the male germ cells [47]. These authors were, however, unable to detect Cx31 mRNA and protein in Sertoli cells. The role of Cx31 in the formation of heterocellular junctions between Sertoli cells and spermatogonia or early spermatocytes is today unclear since it has been previously demonstrated that, in HeLa cells, this Cx was unable to associate with other Cxs present within the seminiferous tubule [48]. RT-PCR analysis of partially purified testicular cells indicates that Cx50 mRNA could be limited to germ cells: pachytene spermatocytes and round spermatids but the tissular distribution of Cx50 protein was not investigated [19]. Thus, it is possible that Cx31 and Cx50 exert specific function(s).

To date, there is little information on the identity of Cxs present in the human testis. Cx43 mRNA and protein are present within the seminiferous tubules and in Leydig cells [30,31]. Cx26 has been characterized within seminiferous tubules [49] and Cx31.9 in testicular vascular smooth muscle [20,50]. By developing a cDNA microarray representing 23,040 genes, Cx40 transcripts were detected in the human testis but no information on the precise localization of Cx40 protein was given [51]. At least, no orthologue of the rat specific testis Cx33 was demonstrated in human [8,44]. However, the lack of Cx33 orthologue in man does not preclude that another member of the Cx family might exert in human similar effects than those reported by Cx33 in the rodent testis [43].

The presence of Cxs or of gap junction proteins homologous to invertebrate, innexins, has been also described in the testes of other species such as frog [52], fish [40,53] and *Drosophila* [54].

3.2. Gap junctions and connexin43 expression in the interstitial compartment

Gap junctions are also present in the interstitial compartment between Leydig cells. Gap junction coupling occurs between freshly isolated mouse Leydig cells and in the TM3 Leydig cell line as demonstrated by dye coupling analysis and electrophysiological measurements [55,56]. In contrast to the seminiferous epithelium in which many Cxs were found, Cx43 was the only Cx detected in Leydig cells of different species such as rat, mouse, guinea pig, mink, frog and human [26,28,29,32,52,55]. In addition, Cx37 was present in the interstitial compartment but specifically located in the endothelial cells of the blood vessels [26].

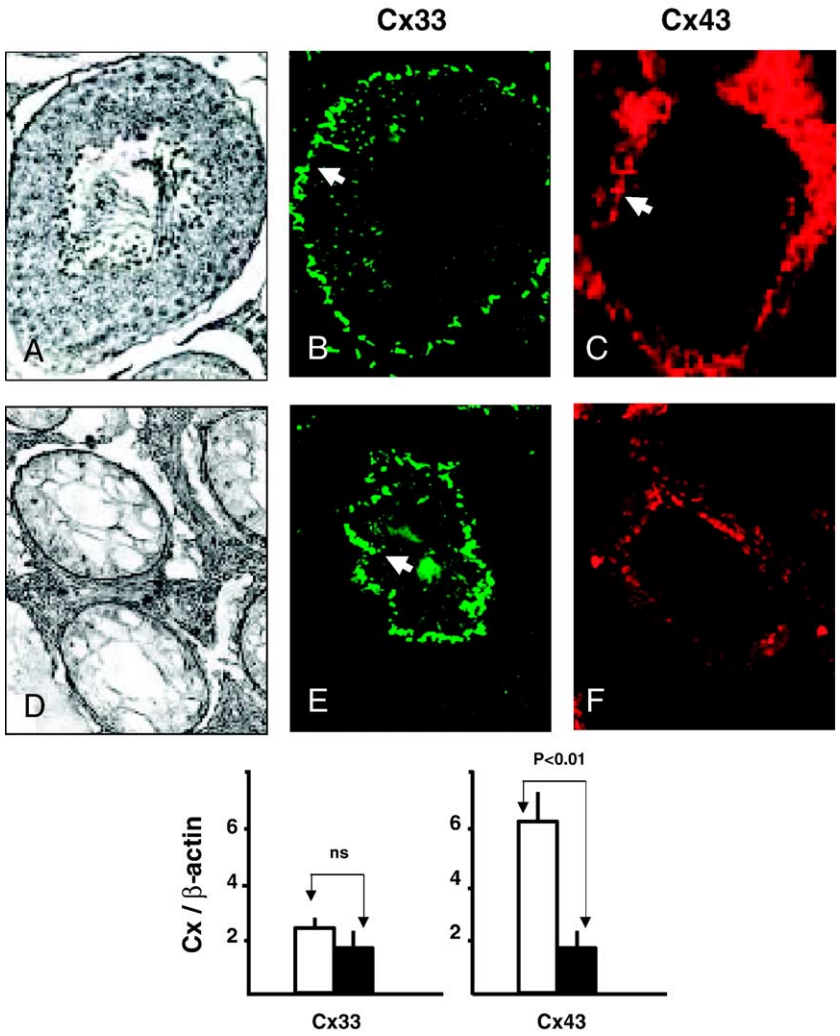

Fig. 2. Analysis of Cx33 and Cx43 expressions within the seminiferous tubules of a mutant cryptorchid rat. (Upper panel) Immunolocalization of Cx33, green fluorescence (B and E) and Cx43, red fluorescence (C and F) in the control scrotal (B and C) and abdominal cryptorchid (E and F) testes of the mutant rat. Histological analysis confirms the lack of germ cells within the seminiferous tubules of the abdominal testis (D) as compared to the control testis (A). (Lower panel) Cx33 and Cx43 mRNA measured by RT-PCR in control (white columns) or abdominal testes (black columns) of the mutant rat. The amounts of amplified products were quantified for each sample by densitometric analysis and results were expressed as the average ratios of the relative optical densities (ROD) of Cx33 or Cx43 PCR products to that of β-actin as previously described [31]. Values are the mean ± S.E.M. of five densitometric readings in five separate experiments. A one-way analysis of variance and a Student's *t* test were used for comparisons between groups. Means were considered significantly different for $P < 0.05$.

3.3. Connexins and the initiation of spermatogenesis

In the testis, there is now clear evidence that Cx gene expression may be related to the development and to the stages of the seminiferous epithelium, characterized by the association of specific germ cells. Cx gene expression profiles in fetal and adult testes, studied by serial analysis of gene expression (SAGE), revealed that Cx43 transcripts were present in Sertoli cells of the fetal testis but at a higher frequency in adult animals [57]. It has been reported, by cDNA microarray, that Cx26 and Cx40 expressions were up-regulated from primitive type A spermatogonia to type B spermatogonia, whereas Cx26 was down-regulated from type B spermatogonia to preleptotene spermatocytes [58]. The reasons for these changes in Cx gene expression during testicular development and between spermatogenic stages are presently unclear.

Within the seminiferous epithelium, immunoreactive Cx43 has been detected between Sertoli and germ cells in newborns and at the time of initiation of spermatogenesis [32,59,60]. During testicular maturation, the signal shifts from the apical to the basal regions of seminiferous tubules in rat [41] and mouse [32,61].

One of the most important events of Cx43 appearance when spermatogenesis takes place is the concomitant location with tight junction molecules preceded by focal adhesion. Generally, there is evidence that adhesion molecules are prerequisite for gap junction formation since it has been reported that the addition of N-cadherin antibodies to Novikoff cell culture blocks the gap junction appearance [62]. In the testis, adhesion molecules such as N-cadherin and α-catenin appear before gap junctions can be detected [63]. By using a Sertoli cell line that expresses Cx43 [64,65], we reported that adhesion molecules (N-cadherin and α-catenin) were first distributed in the

cytoplasm and secondly as dots at the plasma membrane (data not shown). Cx43 was later detected in the cytoplasm of these cells. Then, concomitantly, tight junction molecules and Cx43 were distributed at the cell–cell contact of adjacent cells. Such progressive reorganization of testicular Cx43 is probably under the control of the Sertoli cytoskeletal elements as noticed in other cell types [66]. Altogether these data emphasized that Cx43 appears early during spermatogenesis and that this process follows a precise sequence of events, in relation with its partner molecules.

Another interesting feature is that the levels of Cx43 protein increased during the first wave of spermatogenesis when specifically measured within the seminiferous tubules (Fig. 3). In contrast, the expression of another component of the junctional complex, such as occludin, which is known to participate in the formation of tight junctions (reviewed in Ref. [6]) was not markedly altered during testicular development.

3.4. Control of connexin expression in the testis

The majority of the hormonal factors control the function of their respective tissue or cellular targets in part through gap junction modulation. Such process occurs in the endocrine, immunological and nervous systems: TSH for thyroid; T_3, T_4 for liver; epinephrine for heart; oxytocin for uterus; LH and FSH for ovary (see for review [67]). In the testis, despite the well-documented presence and indispensability of several Cxs, little is known on the physiological effectors capable of regulating cell–cell communication and the steps that are potential sites of the control of gap junctions.

There is now clear evidence that Cx43 mRNA and protein expressions are dependent upon the stages of spermatogenic cycle and that the higher levels are detected at stage VII compared to other stages in rat and mouse testes [26,28,29,32,41,65]. This stage-dependent expression of Cx43 has been also described in the human testis [30,31]. Analysis of Cx43 protein in testes of mutant mice with impaired spermatogenesis led to suggest the existence of a close relationship between the presence of germ cells and Cx43 and the possibility that a specific germ cell population, such as late spermatids, control Cx43 in Sertoli cells [28]. However, the exact mechanisms have not yet been elucidated.

Gap junctions between Sertoli cells, between Sertoli and germ cells, and between Leydig cells may be subject to endocrine and paracrine controls. However, limited information is available on the identity of the factors that control Cx gene expression in the testis. There is evidence that gap junction coupling between Sertoli cells in primary culture is up-regulated by Follicle stimulating hormone, FSH [68]. Its second messenger cAMP is also capable of potentiating GJIC and of altering Cx43 levels [65]. The electrical coupling measured in the presence of cAMP was inhibited when intact seminiferous tubules or isolated cells were incubated in a Ca^{++}-

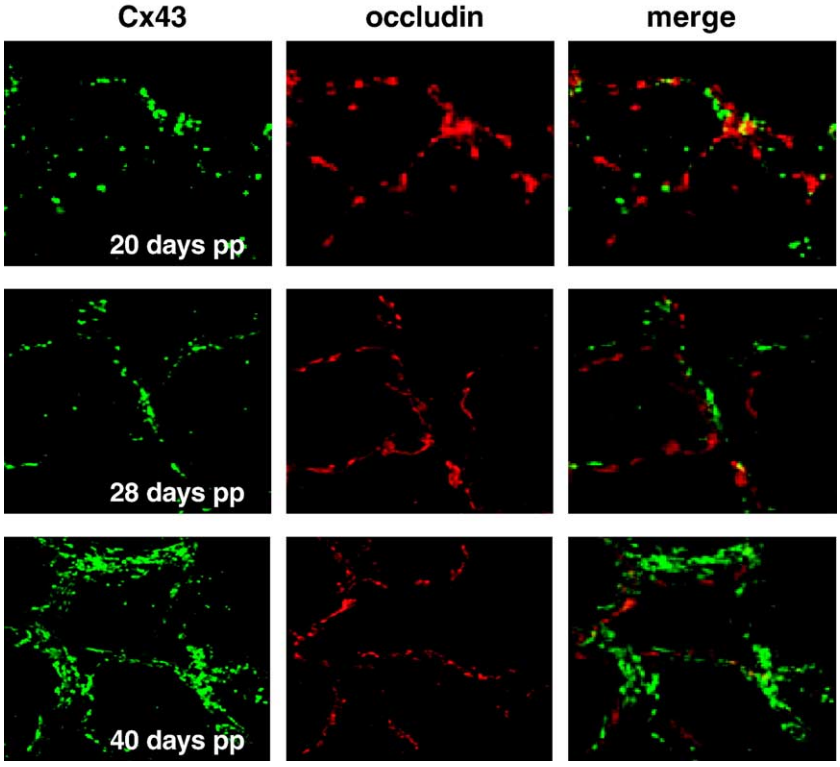

Fig. 3. Immunodetection of Cx43 (a marker of gap junctions) and of occludin (a marker of tight junctions) within seminiferous tubules during rat testicular maturation. At stages of 20, 28 and 40 days of development, testes were fixed, cut and Cx43 (green fluorescence) and occludin (red fluorescence) were immunodetected with specific antibodies as reported [28]. Note that the immunosignal for Cx43 markedly increased from day 20 to day 40 while the intensity of the occludin signal does not show any change during the same developmental period.

free medium suggesting that Ca^{++} is necessary for cAMP responsiveness [69].

The possibility that steroids can control GJIC between adjacent Sertoli cells has been considered. Indeed, esterified forms of testosterone and of 17β-estradiol, and at a lower level, the nonesterified sexual steroids, markedly down-regulated the electrical and diffusional couplings between rat Sertoli cells in primary culture [70,71]. From these results, it has been suggested that lipophilic molecules, such as steroids, could disturb the function of membrane proteins and that, among them, Cx could be one of the molecular targets. A direct interaction of the steroid with the proteolipidic membranous structure and a modification of the Ca^{++} signal between Sertoli cells in response to testosterone, is in agreement with such an hypothesis [72].

Plasmalogens, the major mammalian ether lipids (EL) analogous to cholesterol, are known to control diverse physiological processes such as modulation of membrane fluidity, participation in signal transduction, facilitation of membrane fusion [73]. Recent findings indicate that inactivation of ether lipid biosynthesis in mutant mice caused male infertility and down-regulated Cx43 suggesting that ELs can affect the control exerted by gap junctions on spermatogenesis [74]. The exact mechanism(s) by which these compounds control Cx43 gene expression is presently unknown.

Thyroid hormones are known to play an important role in testicular development and function and exert a specific influence on Sertoli cell proliferation and differentiation during the neonatal period [75]. It has been shown that propylthiouracil (PTU), which is known to induce neonatal hypothyroidism in rats, led to a delocalization of Cx43 from the cell plasma membrane to the cytoplasm of Sertoli cells without altering Cx43 mRNA and protein levels in PTU-treated rat testis [76]. From these data, it has been postulated that, in Sertoli cells, thyroid hormones mainly control Cx43 intracellular trafficking rather than its transcription as previously reported in rat liver epithelial cells [77,78].

Retinoids, which are known to be implicated in cell cycle regulation and cell–cell interaction, are essential for both normal growth and differentiation of germ cells and maintenance of spermatogenesis in the mammalian testis (reviewed in Ref. [79]). We reported that testicular Cx43 expression was reduced in transgenic mice invalidated for the retinoid X receptor β ($RXR\beta^{-/-}$), which show Sertoli cell dysfunction. From these observations, we concluded that retinoids through RXRβ receptors could participate in the control of Cx43 gene expression in Sertoli cell [32]. Such a hypothesis has been validated by a recent findings indicating that vitamin A treatment restored testicular Cx43 expression in a vitamin A-deficient rat model with impaired spermatogenesis [80]. There is also evidence that in many tissues, Cx43 expression is under the regulation of transcriptional factors such as the Fos and Jun subfamilies [81]. Such a regulation probably occurs in the testis, since we reported that jun-d null mice exhibited simultaneously altered spermatogenesis and a marked reduction or abolishment of Cx43 within the seminiferous tubules [28].

Leydig cells, the somatic cells present in the interstitial compartment and responsible of androgens synthesis, responded in vitro to human chorionic gonadotropin (hCG) exposure by reducing Cx43 mRNA and protein levels and inducing a cellular redistribution of the protein [82]. Activators of protein kinase A (PKA) and protein kinase C (PKC) similarly affected Cx43 expression in the TM3 Leydig cell line [83]. In contrast, an inhibitor of protein kinases, staurosporine, was capable to prevent and reverse the uncoupling effect of dibutyryl cAMP and 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) suggesting that cell–cell communication between Leydig cells is down-regulated by both PKA and PKC, interacting in a complex manner [56]. As reported above for Sertoli cells, Cx43 expression in Leydig cells, also appears regulated during fetal and post natal development in an age- and function-dependent manner [59,60]. In the interstitial compartment, the relative temporal correlation between the increase in the number, the size of Leydig cells as well as their Cx43 expression during neonatal life is consistent with the possibility that Cx43 participates in the control of developmental processes involved in testosterone production and secretion [59]. Between mature Leydig cells, Cx43 based gap junctions might locally coordinate the androgenic activity in relation with the stages of spermatogenesis [26].

3.5. Role and functional significance of gap junction and connexins in the testis

There are many indirect observations in support of a role of Cx43 in the control of spermatogenesis. (1) Cx43 forms intercellular contacts between Sertoli cells and proliferating germ cells suggesting that Cx43 participates in specific spermatogenic steps [38]. (2) Cx43 levels are reduced or undetectable within the seminiferous tubules of mutant rat and mice with impaired spermatogenesis [28,31]. (3) Cx43 expression and GJIC are controlled in a stage-dependent manner indicating the existence of a close relationship between the association of specific germ cells and Cx43 expression within the seminiferous tubules [26,28,32,41].

The direct demonstration of Cx43 involvement in spermatogenesis derives from data in Cx null mutant mice (Table 2). Although Cx43 deficient mice die at birth of cardiac malformation [84], it has been reported that testes from null mutant fetuses exhibited a 50% depletion in primordial germ cells indicating that Cx43 participates in the control of germ cell proliferation during fetal development [85]. Similarly, testes from Cx43 null mutant fetuses did not allow normal proliferation and differentiation of germ cells when grafted under the kidney capsules of adult males [86]. The essential role of Cx43 in spermatogenesis comes from original data in which the coding sequence of the Cx43 gene was substituted by coding sequences of Cx32 or Cx40 [87]. These two lines of knock-in mice were viable but the males Cx43KI32 and Cx43KI40 were sterile, exhibiting a Sertoli-cell-only (SCO) phenotype, with seminiferous tubules totally depleted in germ cells. The detailed mechanism(s) that conduces to sterility of

Table 2
Analysis of male fertility in Cx-knock out and Cx-knock in mice

	Consequences on spermatogenesis	References
KO Cx26	Unknown since embryonic lethality	[89]
KO Cx31	No effect on spermatogenesis	[90]
KO Cx32	No effect on spermatogenesis	[91]
KO Cx33	Unknown (embryonic lethality?)	
KO Cx37	No alteration of spermatogenesis but female are sterile	[98]
KO Cx40	No alteration of spermatogenesis	[92–94]
KO Cx43	Germ cell deficiency in fetal testis and in postnatally grafted testis	[84,85]
KO Cx46	No alteration of spermatogenesis	[95]
KO Cx47	No alteration of spermatogenesis	[96]
KO Cx50	No alteration of spermatogenesis	[97]
Cx43 KI 32	Alteration of spermatogenesis	[87]
Cx43 KI 40	(Sertoli-cell-only syndrome)	
Double KO Cx37/Cx40	impaired testicular function due to testicular vascular anomalies	[99]
KO Innexin 4	Reduced number of spermatogonia in Drosophila	[54]

Seminiferous tubules showing Sertoli-cell-only syndrome are totally devoid of germ cells. In mice Cx43KI32 and Cx43KI40, the coding sequence of the Cx43 gene is substituted by the coding sequences of Cx32 or Cx40.

male mice in the two lines are still unknown. For White and Bruzzone, a lack of the substitute Cx to structure gap junction channels with other Cxs, the establishment of impaired communication between Sertoli and germ cells and/or changes in signaling molecules that pass through these heterotypic channels or in the gating properties of these channels, could be responsible for this defect [88]. The essential role of gap junction proteins in spermatogenesis has been also supported by other studies in invertebrates. It has been reported that *Drosophila* males carrying a null mutation in the zero population growth (zpg) locus that encodes a germ line-specific gap junction protein (innexin 4) were sterile with hypotrophic testes that contain reduced numbers of early germ cells: stem cells or early spermatogonia [54].

In contrast to Cx43, knock-out mice for other Cxs did not show testicular abnormalities. Mice homozygous for targeted deletion of Cx26, Cx31, Cx32, Cx40, Cx46, Cx47 and Cx50 genes showed normal fertility [89–97]. Although Cx37-deficient mice were infertile because of abnormal development of both ovocytes and ovarian follicles, $Cx37^{-/-}$ male mice exhibited normal spermatogenesis [98]. Targeted double Cx mutants defective in Cx37 and Cx40 displayed impaired testicular function with spermatogonia vacuolar degeneration and necrosis of the testicular tissue [99]. However, these testicular defects in $Cx37^{-/-}$ $Cx40^{-/-}$ mice were due to testicular vascular anomalies rather than direct alteration of spermatogenesis.

Gap junctions may be implicated in different testicular functions. During fetal testicular development, the possibility that Cx43 may be required for germ cell migration and targeting to the gonads has been suggested in light of data demonstrating a role of Cx43 in the migration of other cell types [100,101]. Whether such specific effects request cell–cell coupling via gap junctions or is independent of this classical role of Cx in the formation of intercellular channels need to be explored [102–104]. During the first weeks of postnatal life, it is tempting to speculate that Cx43 is implicated in the thyroid hormone control of Sertoli cell proliferation [76]. During adulthood, a potential role of gap junctions in maintaining normal germ cell proliferation may be hypothesized since testicular microinjection of oleamide, a well-known inhibitor of GJIC, significantly reduced the number of germ cells within seminiferous tubules of the injected testis (Fig. 4). Whether this effect results from altered synchronization of testosterone synthesis by Leydig cells or from disruption of homocellular and/or heterocellular gap junctions within the seminiferous epithelium or both remain to be clarified. In light of immunological and physiological studies, there is now evidence that Cx43 gap junctions may form a transversal and longitudinal intercellular communication network, corresponding to the initial syncitium-like organization within seminiferous tubules, which allows the coordination of Sertoli cell metabolism. Sertoli cells, through intercellular channels, would ensure metabolic and signaling coupling to germ cells and allow the synchronization of male germ cell proliferation and differentiation [37,38]. This possibility could be considered in light of data in the cerebral cortex where gap junctions are implicated in the regulation of neural progenitor cells proliferation and differentiation during development [105].

3.6. Effect of toxicants on GJIC and connexins in the testis

Within the seminiferous tubules, Sertoli cells are the primary cellular targets for toxicants that may affect multiple functions of this cell type [106]. Thus, the possibility that Cxs could be one of the molecular targets for the deleterious effects of compounds which affect spermatogenesis has been

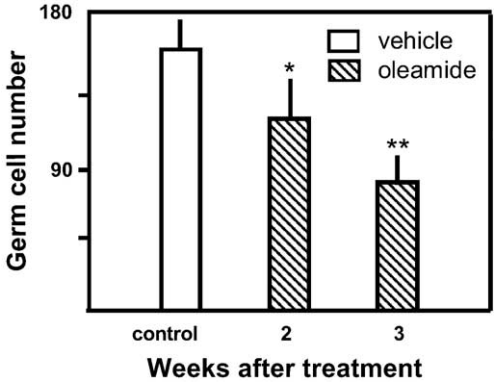

Fig. 4. Effect of oleamide, a blocking agent of GJIC, on germ cell proliferation. Oleamide (50 μM) diluted in DMSO was microinjected in the testes of 5 mature rats (90 days old). For each animal, the contralateral testis was injected with the vehicle alone and used as control. Control and treated testes were embedded in paraffin and cut to perform 5 μm sections. Tissue sections were prepared for fluorescence observations with Vectashield and DAPI mounting medium after hydration of the slides. The number of germ cells, identified by DAPI staining, was quantified using Visilog 5 software (Noesis) in 30 seminiferous tubules two and 3 weeks after microinjection. For each testis, 100 sections were examined. A one-way analysis of variance and a Student's t test were used for comparisons between groups. $*P<0.05$, $**P<0.01$ as compared to testes injected with the vehicle alone.

hypothesized. Gossypol, a potent male non-steroid contraceptive agent, impaired both junctional permeability for fluorescent dyes and junctional electric conductance in primary culture of Sertoli cells [107]. Environmental chemicals, such as ethane 1,2-dimethane sulphonate (EDS), hexachlorocyclohexane (lindane), dichlorodiphenyltrichloroethane (DDT), 1,3-dinitrobenzene (DNB), alkylphenols also affected Cx43 mRNA and protein levels in Sertoli cells of different species [52,108–110]. Other chemical compounds with known estrogenic effects were able to reduce immunoreactive Cx43 levels in Sertoli and Leydig cells [111]. For these authors, Cx43 might serve as a marker gene to identify chemicals with estrogenic effects in the testis. The detailed mechanism(s) whereby these different testicular toxicants affect gap junctional coupling and germ cell proliferation are not entirely known. A reduced phosphorylation of Cx43 linked to an inhibition of apoptosis in Sertoli cells exposed to phthalates have been hypothesized for explaining the inhibition of GJIC [112]. We demonstrated that lindane impaired GJIC by inducing concomitantly Cx43 phosphorylation and ERK activation, and by promoting Cx43 delocalization from the cell membrane to the cytoplasm [35,108,109]. Defects on Cx43 gap junction could also be mediated through inhibition of the p38-MAPK pathway as reported in a murine Sertoli cell line (TM4) exposed to alkylphenol [113].

3.7. Gap junction and connexin expression in human testicular diseases

Previous morphological studies reported severe alterations of testicular gap junctions in human pathological testes with impaired spermatogenesis. By means of freeze-fracture analyses, no gap junctions were found between Sertoli cells in feminized testis, but these membranous structures were frequently observed between Leydig cells [114]. Other authors described the presence of atypical gap junctions in the seminiferous tubules of infertile azoospermic and oligospermic patients [115]. In two other studies, it has been reported that the number of gap junction-like cell membrane specializations was reduced in hypo- or aspermic patients [116] and that these structures were totally affected in men with seminiferous tubules exhibiting SCO phenotype [117]. Structurally normal gap junctions are observed in testes of children aged between 5 and 12 years affected by leukaemia and chemotherapy does not affect the morphology of Sertoli cell contacts [118].

Data which report altered Cx expression in pathological human testes are summarized in Table 3. In patients with SCO syndrome, Cx43 protein was undetectable within seminiferous tubules [30]. Our recent findings indicate that the disappearance of Cx43 protein within the seminiferous tubules of these patients was concomitant with a decrease in testicular Cx43 mRNA levels while Cx43 transcripts and protein were maintained in Leydig cells [31]. These data suggested that Cx43 alteration, which was probably due to a defect in the maturation of Sertoli cells, could be used as a biological marker of Sertoli cell dysfunction [31,61].

Impaired GJIC and Cx dysfunction are currently associated with the effects of carcinogens and oncogenes [119]. It has been reported that this feature is typical of numerous neoplastic tissues (reviewed in Ref [33,120,121]). By means of immunohistochemistry, Brehm et al. demonstrated that Cx43 was absent in human testis infiltrated with carcinoma-in-situ or in seminoma [49] while its presence was evidenced in normal control testis [30,31]. In other studies, Cx43 mRNA and protein were normally detected in a group of pure testicular seminoma and in a seminoma cell line [33,122]. However, fine immunolocalization analysis indicated that Cx43 was aberrantly detected within the cytoplasm of seminoma cells instead to be localized, at the cell plasma membrane level. In transgenic mice, which develop Leydig cell tumors, we observed reduced testicular Cx43 levels and aberrant localization of the protein [123]. Analysis of the kinetic of Cx43 behavior during early and advanced stages of Leydig cell tumorigenesis allowed us to propose Cx43 delocalization as an early event of uncontrolled cell proliferation in the pathological testis. In contrast to Cx43, which was dramatically affected, two other Cxs (Cx26 and Cx40) were up-regulated in tumoral testicular tissues. Cx26 was undetectable in normal human testis but displayed a strong intracytoplasmic Sertoli cell staining in infiltrated tubules with spermatogonial arrest or CIS-only [49]. It is interesting to parallel this observation with previous data in human breast showing that Cx26 expression was strengthened in proportion to the grade of malignancy [124]. In a recent study, it has been reported that Cx40 mRNA levels increased in testicular seminoma as compared to normal human testis [51]. Although the reasons for the alteration of Cx26 and Cx40

Table 3
Diseases of the male genital tract associated with altered expression of Cxs

Diseases		Connexin	References
Secretory azoospermia (spermatogenic arrest)	Sertoli cell only syndrome[a]	Cx43	[31]
Testis cancer	CIS, seminoma, seminoma cell line	Cx26, 43	[33,49,122]
	Leydig cell tumor and tumoral Leydig cell line[b]	Cx43	[123]
Prostatic tumor	Benign prostatic hyperplasia	Cx43, 32	[141]
	Adenocarcinoma	Cx26, 32, 43	[132,133,141,146]
	Malignant prostate epithelial cell lines[c]	Cx43, 32	[131,139,140,142,143,146]

[a] Sertoli cell syndrome is characterized by seminiferous tubules, totally depleted in germ cells, in which only Sertoli cells are present.
[b] BLT-1 murine Leydig tumor cell line.
[c] PC-3, LNCap, DU-145, ALVA-31, TSU-Pr1 and RWPE-2 human prostate cancer cell lines.

expressions in tumoral testes are presently unknown, the possibility that these two Cx would be considered as potential new diagnostic markers for testicular germ cell tumors has been hypothesized.

4. Gap junction and connexin expression in the epididymis and seminal vesicles

4.1. Epididymis

The formation of mature germ cells involves not only their proliferation, meiosis and differentiation during spermatogenesis within the seminiferous tubules but also their final maturation during transit in the initial segment, the caput, the corpus and the cauda of the epididymis. In the epididymal tubule, spermatozoa acquire both fertilizing capacity and progressive motility. The presence and the physiological role of gap junctions in these latter processes are still poorly understood.

Gap junctions were first identified by freeze fracture electron microscopy between adjacent epididymal principal cells at their apical and lateral margins [125,126]. Immunoreactive Cx43 based gap junctions were localized between principal and basal cells but not between adjacent principal cells [127]. More recently, by using RT-PCR and restriction enzyme mapping analyses, the presence of multiple Cx mRNAs (Cx26, Cx30.3, Cx31.1 and Cx32) were reported in the rat epididymis [128]. In addition, these authors demonstrated that the expression of these Cxs was segment-specific, age-dependent and that their localization varied within the tissue. The possibility that Cx26 and Cx43 could play a major role during differentiation of the epididymal epithelium and that GJIC could be crucial to epididymal function has been suggested [129].

There is evidence that differential regulation of mRNA or protein levels occurs along the epididymis. In the adult rat epididymis, Cx43 expression was androgen-dependent in the initial segment of the epididymis [127] and thyroid hormone-dependent in the proximal regions of the epididymis but not in the cauda epididymidis [76]. The reasons why androgens and thyroid hormones preferentially regulate Cx expression in different regions of the epididymis are presently unknown.

4.2. Seminal vesicles

The seminal vesicles join the ampulla of the deferens to form the beginning of ejaculatory ducts. Histologically, seminal vesicle epithelium is constituted by highly specialized columnar cells demarcated from its attached stroma (primarily smooth muscle). Seminal vesicles secrete a great variety of products that are essential in male fertility. To date, only one report demonstrates the presence of Cx32 mRNA and protein in seminal vesicles of adult rats and the absence of Cx43 and Cx26 in this exocrine gland [130]. However, the precise cellular localization of Cx32 and its physiological role were not yet determined.

5. Gap junction and connexin expression in the prostate

5.1. Connexins in the prostate

In the prostatic epithelium, gap junctions which are present between secretory luminal and basal cells can coordinate the activities between the two cell types [130]. Cx32, Cx40 and/or Cx43 were identified as major constituents of gap junctions in prostate tissue and in primary prostate epithelial cells. In the adult rat prostate, Cx32 and Cx26 were found on secretory epithelial cells, but Cx43 was not detected [130]. Reports on analysis of Cx expression in normal adult human prostate are controversial. While some investigators found Cx32 and no Cx43 expression [131,132], others reported the presence of Cx43 in benign prostate cells [133]. There is evidence that Cx43 is expressed in undifferentiated and mature basal cells as well as urogenital sinus mesenchymal cells, whereas Cx32 is expressed by differentiated prostate epithelial cells [134]. For both epithelial and mesenchymal cells, the “switch” from Cx43 to Cx32 expression appears to be associated with prostatic differentiation. Immunohistochemical analysis revealed that Cx26 was detected not only between adjacent basal and adjacent luminal cells but also between basal and luminal cells. In contrast, Cx43 was localized between adjacent basal cells and between basal and luminal cells but not between adjacent luminal cells [135]. The presence of Cx43 has been also recently demonstrated in interstitial cell of the prostate [136]. For these authors, interstitial cells could function as pacemaker cells generating slow wave potentials mediated through Cx43 gap junctions and conducting to the smooth muscle tone of the prostate.

5.2. Hormonal control of connexins in the prostate

It is well recognized that in steroid sensitive organs, Cx expression is regulated by steroid hormones including androgens and estrogens. Recent data showed that castration was associated with both a marked increase in Cx43 expression and an induction of apoptosis in the prostate gland cells and that the treatment of animals with testosterone or DHT abolished the castration-induced Cx43 expression and prevented apoptosis [137]. Thus, it has been postulated that Cx43 might participate in prostate involution. The findings that Cx32 was not affected in the same condition by androgens led to suggest that Cx43 might mediate some of the critical aspects of prostate epithelial cell survival and proliferation. Since no androgen-responsive element was identified in the Cx43 gene, it is possible that the transcriptional effect of androgens on Cx43 gene could be mediated through AP, cAMP and Sp sites that are present in the Cx43 promoter. There is also strong evidence that estrogens control Cx expression in prostate tissue. Indeed, alteration in estrogen receptors was associated with impaired expression of gap junction molecules [138]. Neonatal estrogenization of the prostate increased Cx43 and decreased Cx32 expressions that may result in defective cell–cell communication [134]. For these authors, the altered ratio Cx43/Cx32 could reflect a “switch” from differentiation to dedifferentiation of prostate

epithelial cells [134]. Such alteration of Cx43 and Cx32 levels, associated with altered GJIC, has been also reported in the non-tumorigenic human prostate epithelial cell line after exposure to estrone [139,140]. Whether these effects are classically mediated through estrogen response element or AP-1 site, or imply activation of cAMP-dependent signal transduction pathways remains to be elucidated.

5.3. Connexins and prostatic diseases

Like the majority of neoplastic cells, prostate tumor cells exhibit impaired GJIC. In contrast to human benign prostatic hyperplasia, reduced Cx43 and Cx32 expressions were reported in prostate carcinoma cells (Table 2) [133,141,142]. The altered Cx43 and Cx32 expressions in prostate cancer samples might lead to aberrant and uncontrolled epithelial growth and probably have a role in dedifferentiation and tumor progression. As prostate tumor progress to more undifferentiated stages, Cxs were aberrantly localized in the cytoplasm and a loss of Cx expression was observed in advanced stages of carcinoma [132]. Studies with human prostate cancer cell lines revealed that the major cause of the altered GJIC in these cells was due to impaired trafficking of Cxs rather than their inability to form gap junction [143]. By using nontumorigenic and tumorigenic human prostate epithelial cell lines, it has been reported that an increase in the Cx43/Cx32 ratio might restore GJIC in junctionally-deficient cells providing a basis for the development of new strategies for the prevention and treatment of human prostate cancer [139,140,144]. Similarly, forced expression of Cx26 on three human prostate cancer cell lines led to an inhibition of tumoral cell growth that may be reinforced by combining gene therapy with chemotherapy [145]. Recently, a novel mechanism for adhesion regulation by a gap junction-independent Cx26 function that correlates with prostate disease progression has been described [146].

6. Gap junction and connexin expression in the Corpus cavernosum

Modulation of smooth muscle tone, through myocyte contraction or relaxation, is critical for maintaining the physiological functions of diverse vascular and non-vascular organs of the body. The penis is a vascular organ and changes in the contractile status of corporal and arterial govern the flow of blood to and from the penis and have a major impact on erectile capacity. In concert with the autonomic nervous system and myogenic intracellular signal transduction mechanisms, the regulation of this process is dependent on the presence of gap junctions, between corpus cavernosum smooth muscle cells, that allow the formation of a syncytial cellular network coordinating uniform corporal smooth muscle tone. Gap junctions and the major component of these structures, Cx43, have been characterized in situ and in cultured corporal smooth muscle [147–152].

Emerging evidence suggests that, outside impairment of K^+ channel activity in cavernosal and arterial smooth muscle cells, a reduced passive conductance of electrical signals due to gap junction impairment, could be responsible to erectile dysfunction (reviewed in Ref. [153]). Although a marked increase in Cx43 mRNA levels was reported in corpora tissue of diabetic rats [154] and a negative correlation was observed between Cx43 levels and patient age [155], there is today evidence that gap junctions in the penis are not directly influenced by erectile diseases and that Cx43 is constitutively synthesized in this tissue [156].

7. Conclusions and perspectives

Gap junctions and Cxs appear as a constant in the male reproductive tract and can play a major role in several functions including germ cell proliferation and differentiation, and in modulating the initiation and maintenance of smooth muscle tone in different structures of the male tract. Although there is at present no direct demonstration that impaired Cx expression may be involved in pathogenesis of human male sexual function, it is likely to suspect Cx dysfunction(s) in some testis and prostate diseases (spermatogenic arrest, seminoma, prostatic adenocarcinoma) and in the physiopathology of the human erectile response. The development of strategies to restore normal GJIC in affected cells of the male genital tract will be required after precisely determining the nature of Cx(s) implicated, Cx gene transfer to these cells and/or to develop effective pharmacological agents. Such strategies have been initiated in different domains of male reproductive pathology such as prostate disease [140] and erectile dysfunction [156].

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