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Review

Connexin 43: its regulatory role in testicular junction dynamics and spermatogenesis

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Summary. Spermatogenesis is an intensely regulated process of germ cell development which takes place in the seminiferous tubules of the testis. In addition to known endocrine and autocrine/paracrine signaling pathways, there is now strong evidence that direct intercellular communication via gap junction channels and their specific connexins represents an important mechanism in the regulation of spermatogenesis. Another possibility is that connexins may indirectly regulate the spermatogenic process through modulation of tight and adherens junction proteins, further main structural components of the Sertoli-Sertoli junctional complexes at the blood-testis barrier site. The present review is focused on connexin 43 and updates its possible roles and functions in testicular junction dynamics and in the initiation and maintenance of spermatogenesis. In addition, testicular phenotypes of recently generated (1) conventional connexin 43 knockout mice, (2) connexin 43 knockin mice and (3) transgenic mice exhibiting a cell-specific (conditional) connexin 43 knockout will be discussed.

Key words: Connexin 43, Blood-testis-barrier, Junction dynamics, Spermatogenesis, Testis

Introduction

Spermatogenesis, the entire process of germ cell (GC) development, occurs within the testicular seminiferous tubules, which consist of peritubular tissue and the seminiferous epithelium. The latter is composed of GCs in different developmental stages and the structurally and nutritionally supporting somatic Sertoli cells (SCs). The spermatogenic process can be divided into four phases: (1) the mitotic proliferation and differentiation of diploid spermatogonia, (2) meiotic divisions of tetraploid spermatocytes and (3) the transformation of haploid round spermatids into spermatozoa (spermiogenesis), which (4) are released into the tubular lumen (spermiation) (Bergmann, 2006; Weinbauer et al., 2010). The regulation of this process involves both endocrine effectors (follicle stimulating and luteinizing hormone) and cell-cell interactions mediated through either autocrine/paracrine factors (like testosterone) or direct intercelluar contacts (occluding and anchoring junctions) and communication channels, consisting of gap junctions (GJs) and their constitutive proteins, the connexins (Cxs) (for reviews, see Mruk and Cheng, 2004; Sofikitis et al., 2008; Cheng et al., 2010; Pointis et al., 2010).

At the basal third of the seminiferous epithelium inter-SC tight junctions (TJs) form the anatomical basis of the blood-testis-barrier (BTB). By this, mature SCs segregate the seminiferous epithelium into a basal compartment (containing spermatogonia and preleptotene spermatocytes) and an adluminal compartment (containing the other GCs) (Dym and Fawcett, 1970). Two main functions are postulated for the BTB: (1) creation of an immunological barrier to protect haploid GCs and (2) generation of a specific milieu for GC development (for review, see Byers et al., 1993). Moreover, the BTB is a very dynamic structure undergoing disassembly and reassembly during the passage of developing GCs from the basal to the adluminal compartment (for review, see Mruk and Cheng, 2004).

In general, three types of intercellular junctions can be found in the seminiferous epithelium: (1) the above mentioned TJs, (2) anchoring junctions and (3) GJs. The anchoring junctions can be additionally subdivided into four types: cell-cell actin-based adherens junctions

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(AJs), cell-matrix actin-based focal contacts, cell-cell intermediate filament-based desmosomes and cell-matrix intermediate filament-based hemidesmosomes. Furthermore, there are two specific AJs unique to the testis, such as ectoplasmatic specializations and tubulobulbar complexes (for review, see Cheng and Mruk, 2002; Mruk and Cheng, 2004).

Interestingly, at the level of the BTB, inter-SC TJs constitute, together with AJs (e. g. basal ectoplasmatic specializations) and GJs, the so-called Sertoli-Sertoli junctional complexes (for reviews, see Pelletier and Byers, 1992; Lee et al., 2007; Hermo et al., 2010; Pointis et al., 2010). In this area, the different junctions are highly intermingled with each other and recent data suggest that they could mutually influence the expression and function of one another. Even Cxs, in particular Cx43, are supposed modulators of the other junctions representing another regulatory mechanism of the spermatogenic process. Thus, the present review has been designed to describe the recent progress on testicular Cx43 research with respect to its regulatory roles on other junction types, its relation to normal and impaired spermatogenesis and its role in male fertility.

Molecular architecture of testicular TJs, AJs and GJs

TJs, AJs and GJs perform different functions and are constituted of specialized integral membrane proteins, peripheral adaptors and signaling molecules implicated in sealing (e. g. occludin, claudin-11), adhesion (e. g. N-cadherin, ß-catenin) and communication (e. g. Cx43) between adjacent cells (Fig. 1). Moreover, they share specific adaptor molecules, particularly zonula occludens-1 (ZO-1), by assembling into macromolecular complexes. Via such common adaptors, the underlying cytoskeleton and associated signaling pathways the junctions can interact with each other (for reviews, see Lee and Cheng, 2004; Derangeon et al., 2009).

The various junctional proteins have been extensively reviewed elsewhere (Cheng and Mruk, 2002; Lui et al., 2003; Lee and Cheng, 2004; Mruk and Cheng, 2004; Goossens and van Roy, 2005; Hermo et al., 2010; Pointis et al., 2010) and are not discussed in detail herein. However, for a better understanding this review offers a short overview.

Tight and adherens junctions

Morphologically, TJs form a continuous circumferential seal. To date, three classes of TJ membrane spanning proteins have been found in the testis: occludin, claudins and junctional adhesion molecules (JAMs) (Furuse et al., 1998; Martìn-Padura et al., 1998; Cyr et al., 1999; Meng et al., 2005). Occludin and claudins can be partners in creating sealed TJs, organized into oligomers and hetero-oligomers as functional protein complexes. Seven different claudins (claudin-1, -3, -4, -5, -7, -8 and -11) and two JAMs (JAM-1 and -2) are currently expressed in the testis (for review, see Lui et al., 2003). Various peripheral cytoplasmic adaptors linking TJ integral membrane proteins to the cytoskeleton have also been identified. These include ZO-1 (Byers et al., 1991; Fink et al., 2006), ZO-2 (Jesaitis and Goodenough, 1994; Fink et al., 2006), cingulin (Citi et al., 1988), symplekin (Keon et al., 1996) and others (for review, see Lui et al., 2003).

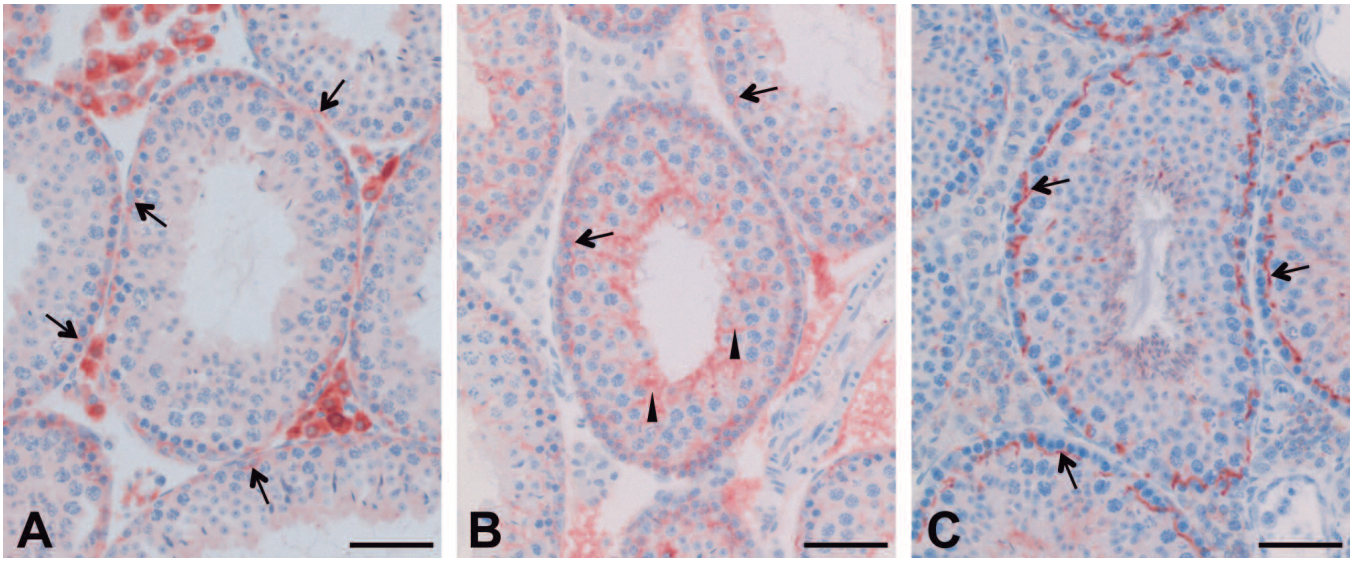

Fig. 1. Occludin **(A)**, N-cadherin **(B)** and Cx43 **(C)** immunohistochemistry of adult WT mouse testes. Note the presence of the three proteins at the level of the BTB (arrows). An N-cadherin immunosignal is additionally detectable at the apical region of the seminiferous tubule (**B**, arrowheads). Cytoplasmic staining in Leydig cells **(A)** derives from unspecific secondary antibody staining. Scale bars: 50 μm.

As above mentioned, the testis possesses unique AJ types such as ectoplasmatic specializations. The transmembrane proteins of cell-cell actin-based AJs are cadherins (e. g. E- and N-cadherin). To date more than 30 cadherins have been identified (for review, see Cheng and Mruk, 2002). Their cytoplasmic carboxy terminus associates with peripheral adaptors, the ß- or γ-catenins (Huber and Weiss, 2001). α-catenin in turn interacts with ß- or γ-catenin, linking this complex directly to the actin cytoskeleton (Rimm et al., 1995) or via its interactions with further adaptors, such as ZO-1, α-actinin or vinculin. In addition to the cadherin/catenin adhesion complex, nectins (e.g. nectin-2 and -3) belong to another family of AJ transmembrane proteins interacting with the peripheral adaptor afadin. As such, afadin can bind the nectin/afadin complex directly to actin or via ZO-1, α-catenin or ponsin (for review, see Cheng and Mruk, 2002).

Gap junctions and connexin 43

In general, GJs allow a direct cell-cell exchange of small molecules (<1 kDa) such as nucleotides, second messengers, peptides and ions (e. g. Ca^{2+}). Gap junctional intercellular communication (GJIC) regulates essential processes during cell proliferation and differentiation, homeostasis and oncogenic transformation (for review, see Kumar and Gilula, 1996). One GJ channel is composed of two hemichannels or connexons, each built up of six Cx protein subunits in the plasma membrane of two neighboring cells (for review, see Bruzzone et al., 1996). The multigene Cx family consists of at least 21 members in humans and 20 members in mice. The current Cx nomenclature is based on their theoretical molecular mass (Söhl and Willecke, 2004). Six identical Cx subunits form homomeric connexons, whereas heteromeric hemichannels are composed of more than one type of Cxs. GJ channels can be homotypic (if connexons are identical) or heterotypic (if the two connexons are different) (Kumar and Gilula, 1996). Although only Cxs have been found in the testis, there exists a second family of GJ proteins which were cloned in vertebrates: the pannexins (Bruzzone et al., 2003). These proteins are homologous to the invertebrate GJ family of innexins (Baranova et al., 2004).

In the testis, al least eleven Cx isoforms (Cx26, Cx31, Cx31.1, Cx32, Cx33, Cx37, Cx40, Cx43, Cx45, Cx46 and Cx50) with distinct distribution patterns have been demonstrated (Risley, 2000). Among those, Cx43 is the predominant Cx in the testis of different species (Risley et al., 1992; Pelletier, 1995; Batias et al., 1999; Steger et al., 1999). In mouse testes, Cx43 is expressed from day 11.5 post coitum to all postnatal stages (Pérez-Armendariz et al., 2001). However, in other species like humans (Steger et al., 1999) its expression seems not to start until the onset of spermatogenesis at puberty. During postnatal testicular development in rodents, the Cx43 immunosignal shifts from the adluminal to the basal region of the seminiferous epithelium (Batias et al., 2000; Bravo-Moreno et al., 2001). In mature testes, Cx43 is mainly found at the level of the BTB between neighboring SCs. Furthermore, it is also located between SCs and specific GCs (spermatogonia and primary spermatocytes) and its expression becomes stage-dependent (Risley et al., 1992; Pelletier, 1995; Tan et al., 1996; Batias et al., 1999, 2000; Steger et al., 1999; Decrouy et al., 2004).

In addition to the classical role of GJs, recent studies reported that undocked connexons and Cxs by themselves also exert physiological but non-junctional roles. For example, connexons can act as transporters between the cell and its extracellular space by releasing adenosine triphosphate, prostaglandin or glutamate (for reviews, see Stout et al., 2004; Spray et al., 2006).

Regulatory roles of Cx43 in spermatogenesis

Many studies now exist supporting functional roles of Cx43 in the regulation of spermatogenesis. However, the accurate mechanisms by which Cx43 and/or its GJs control development of GCs are unknown.

Indirect evidence for regulatory roles of Cx43

Cx43 forms intercellular channels between SCs and proliferating GCs (Decrouy et al. 2004) and its expression is controlled in a stage-dependent manner (Risley et al., 1992; Pelletier, 1995; Tan et al., 1996; Batias et al., 1999, 2000; Steger et al., 1999). These observations indicate a close relationship between specific GC populations and Cx43 synthesis and an involvement of Cx43 in the physiological GC developmental process. Moreover, Cx43 GJs between SCs seem to form an intercellular communication network corresponding to an initial "syncytium-like organization" within the seminiferous epithelium, allowing the tubular coordination of SC metabolism and indirectly, via metabolic and signaling coupling, the synchronization of GC proliferation and differentiation (Risley et al., 2002; Decrouy et al., 2004).

Several knockout (KO) mice (ebo/ebo, jun-$d^{-/-}$, RXRß$^{-/-}$ and mice with mosaic mutation and partial deletion in the long arm of the Y chromosome), which are characterized by the presence of a severe spermatogenic impairment, revealed either a reduced or undetectable testicular Cx43 immunoreaction (Batias et al. 1999, 2000; Kotula-Balak et al., 2007). Similar alterations in Cx43 expression were found in human patients with testicular carcinoma in situ, SC tumor, seminomatous or non-seminomatous GC tumors (Brehm et al., 2002; Roger et al., 2004; Brehm et al., 2006a, b; Steiner et al., 2011) and canine patients with seminoma (Rüttinger et al., 2008). Furthermore, an aberrant cytoplasmic accumulation of Cx43 was shown in human seminoma cells (Mauro et al., 2008). Human patients with Klinefelter's syndrome associated with impaired spermatogenesis (Kotula-Balak et al., 2007) and infertile

men with idiopathic spermatogenic impairment (Steger et al., 1999; Defamie et al., 2003; Matsuo et al., 2007) also showed a reduction or loss of Cx43. In cryptorchid testes of stallions (Hejmej and Bili*f*ska, 2008) and mutant rats (Defamie et al., 2003) a clear reduction or loss of Cx43 expression in SCs was concomitantly observed with a defect in spermatogenesis. Beyond that, treatment of animals with reproductive toxicants like di(2-ethylhexyl)phthalate or bisphenol A also caused spermatogenic impairment and a down-regulation of testicular Cx43 (Salian et al., 2009; Sobarzo et al., 2009). Additionally, several in vitro studies demonstrated that SC junctions at the BTB site, particularly Cx43 based GJs, are early targets for testicular toxicants or phosphodiesterase inhibitors. These substances affected intercellular junctions by either reducing the amount or inducing aberrant intracellular localization of their transmembrane proteins (Defamie et al., 2001; Fiorini et al., 2004; Li et al., 2009a). Altogether, the observed positive correlation between testicular disorders and apparent decrease or loss of Cx43 expression suggests an important role of Cx43 in the control of spermatogenesis.

Direct evidence for regulatory roles of Cx43

Direct observations derive from investigations using transgenic mice, in which the Cx43 gene was either deleted or substituted by another Cx (Table 1). In the last decade, different Cx43 KO and knockin (KI) mice have been generated. Total disruption of the Cx43 gene leads to altered cardiac morphology and sudden perinatal death (Reaume et al., 1995). However, the importance of Cx43 to male gametogenesis has been shown by a 50 % depletion of primordial GCs in fetal male mice lacking the Cx43 gene (Juneja et al., 1999). It was further demonstrated that primordial GCs are already GJIC competent cells and that GC deficiency in Cx43 KO embryos may arise from an increased apoptosis by abnormal p53 activation (Francis and Lo, 2006). A recent *in vitro* study confirms the involvement of Cx43 GJs in GC number regulation by controlling spermatogonia survival rather than proliferation (Gilleron et al., 2009). In Cx43 null mutants, it was additionally shown that the loss of the Cx43 gene exerts profound effects on the expression pattern of other testicular Cxs. In fetal testes of Cx43 KO mice only four Cx mRNAs were detectable (Cx26, Cx37, Cx40 and Cx45) compared to normal fetal wildtype (WT) testes exhibiting eight Cx members (Juneja, 2003). The analysis of testicular development by grafting Cx43 KO testes under the kidney capsules of adult WT mice revealed that the GC deficiency (spermatogenic arrest at the level of spermatogonia or SC-only phenotype) also persists postnatally, suggesting that intercellular communication via Cx43 channels is required for postnatal expansion of the male germ line (Roscoe et al., 2001). However, grafting Cx43 KO testes under the kidney capsules of castrated adult WT males showed that the steroidogenic function of interstitial Leydig cells was not affected by the absence of Cx43 (Kahiri et al., 2006).

Further data concerning functional roles of Cx43 in spermatogenesis derive from two studies in which the Cx43 gene was either substituted by the coding sequences of Cx32 and Cx40 (Plum et al., 2000) or Cx26 (Winterhager et al., 2007). Cx43KI32 and Cx43KI40 mice were viable, but spermatogonial amplification remained defective with an arrest of spermatogenesis at the level of spermatogonia or SC-only syndrome, emphasizing the unique role of Cx43 in spermatogenesis. Similar results were obtained from a more recent study in which the coding region of Cx43 was replaced by that of Cx26. Male mice were viable, but infertile. Spermatogenesis was dramatically impaired leading to the absence of GCs beyond early spermatocytes. In summary, these Cxs cannot functionally replace Cx43 in the seminiferous epithelium.

In order to circumvent known perinatal lethality and pleiotropic effects of the general Cx43 deficiency (Reaume et al., 1995) and to clarify cell specific roles of Cx43 on testicular development and spermatogenesis *in vivo*, a viable conditional Cx43 KO mouse line, which lacks the Cx43 gene solely in SCs (SCCx43KO), was generated by two groups using the Cre/loxP recombination system (Brehm et al., 2007; Sridharan et al., 2007). Adult SCCx43KO$^{-/-}$ mice showed normal testis descent and development of the urogenital tract, but testis size and weight was drastically lower when

Table 1. Direct evidence for regulatory roles of Cx43 in spermatogenesis.

	Consequences on spermatogenesis	References
Cx43 KO	50 % primordial GC depletion in fetal testes due to increased GC apoptosis and postnatal GC deficiency in grafted testes	Juneja et al., 1999; Roscoe et al., 2001; Francis and Lo, 2006
Cx43KI32 Cx43KI40	Arrest of spermatogenesis at the level of spermatogonia or SC-only syndrome; reduced number of spermatogonia	Plum et al., 2000
Cx43KI26	Spermatogenic impairment: presence of only spermatogonia and some spermatocytes	Winterhager et al., 2007
SCCx43KO	In the vast majority of seminiferous tubules: spermatogonial arrest or SC-only syndrome; reduced number of spermatogonia	Brehm et al., 2007; Sridharan et al., 2007

compared with heterozygous and WT littermates. Histological analysis revealed quantitative and qualitative normal spermatogenesis in WT (Fig. 2A) and SCCx43KO$^{+/-}$ mice (Fig. 2B) indicating that one Cx43 allele in SCs is still sufficient to support functional spermatogenesis. In contrast, SCCx43KO$^{-/-}$ mice (Fig. 2C) showed in the vast majority of the seminiferous tubules an arrest of spermatogenesis at the level of spermatogonia or SC-only syndrome, intratubular cell clusters and vacuolated tubules. Thus, the loss of both Cx43 alleles in SCs prevents the initiation of spermatogenesis. Additionally, statistical analysis revealed a reduced number of spermatogonia and increased SC numbers per seminiferous tubule.

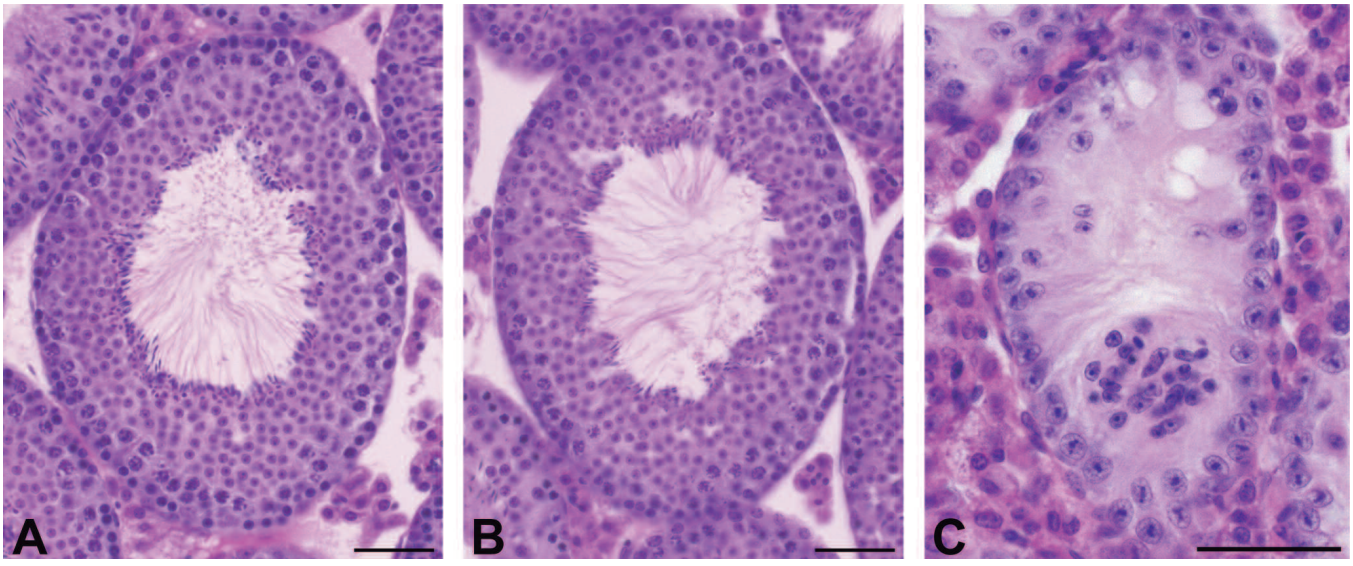

Fig. 2. Hematoxylin-eosin staining of testes from adult WT **(A)**, SCCx43KO$^{+/-}$ **(B)** and SCCx43KO$^{-/-}$ **(C)** mice (Brehm et al., 2009, modified). Tubules of WT (**A**) and SCCx43KO$^{+/-}$ (**B**) mice reveal normal spermatogenesis. In contrast, SCCx43KO$^{-/-}$ animals exhibit smaller tubules with SCO syndrome **(C)** or spermatogenic arrest at the level of spermatogonia (not shown). Scale bars: 50 μm.

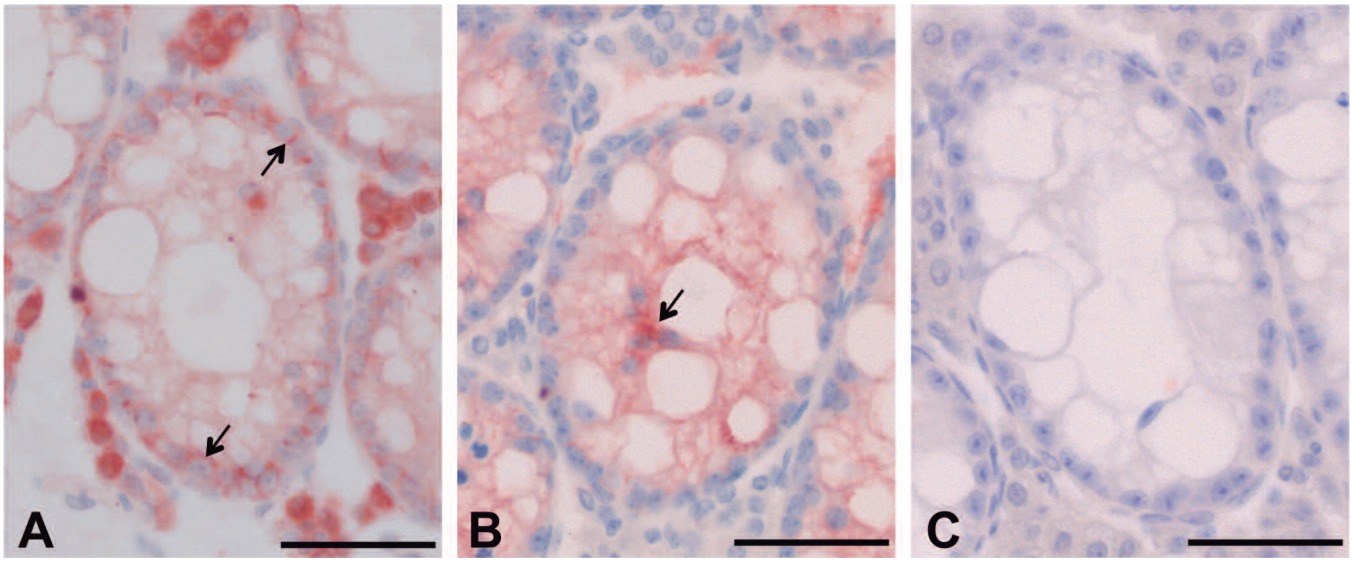

Fig. 3. Occludin (**A**), N-cadherin **(B)** and Cx43 **(C)** immunostaining of adult SCCx43KO$^{-/-}$ mouse testes. Occludin is still localized in the area of the BTB (**A**, arrows). For N-cadherin a diffuse basal and adluminal immunoreaction can be detected, small cell clusters are also positive (**B**, arrow). As expected, there exists no Cx43 immunosignal in the seminiferous tubules of KO mice. Scale bars: 50 μm.

Furthermore, mature SCs were found to be still proliferating (Sridharan et al., 2007), emphasizing the crucial contribution of Cx43 to the maturational progression of SCs, which normally results in the cessation of SC mitosis during puberty. Recent *in vitro* studies also provide evidence for the involvement of Cx43 GJs in the negative control of SC proliferation (Gilleron et al., 2006, 2009).

Cx43 and regulation of other testicular junction types

The accurate mechanisms by which Cx43 regulates the development of GCs are still unknown. One possibility could be that Cx43 and/or its GJ channels control the spermatogenic process through regulation of TJs and AJs via specific peripheral adaptors,

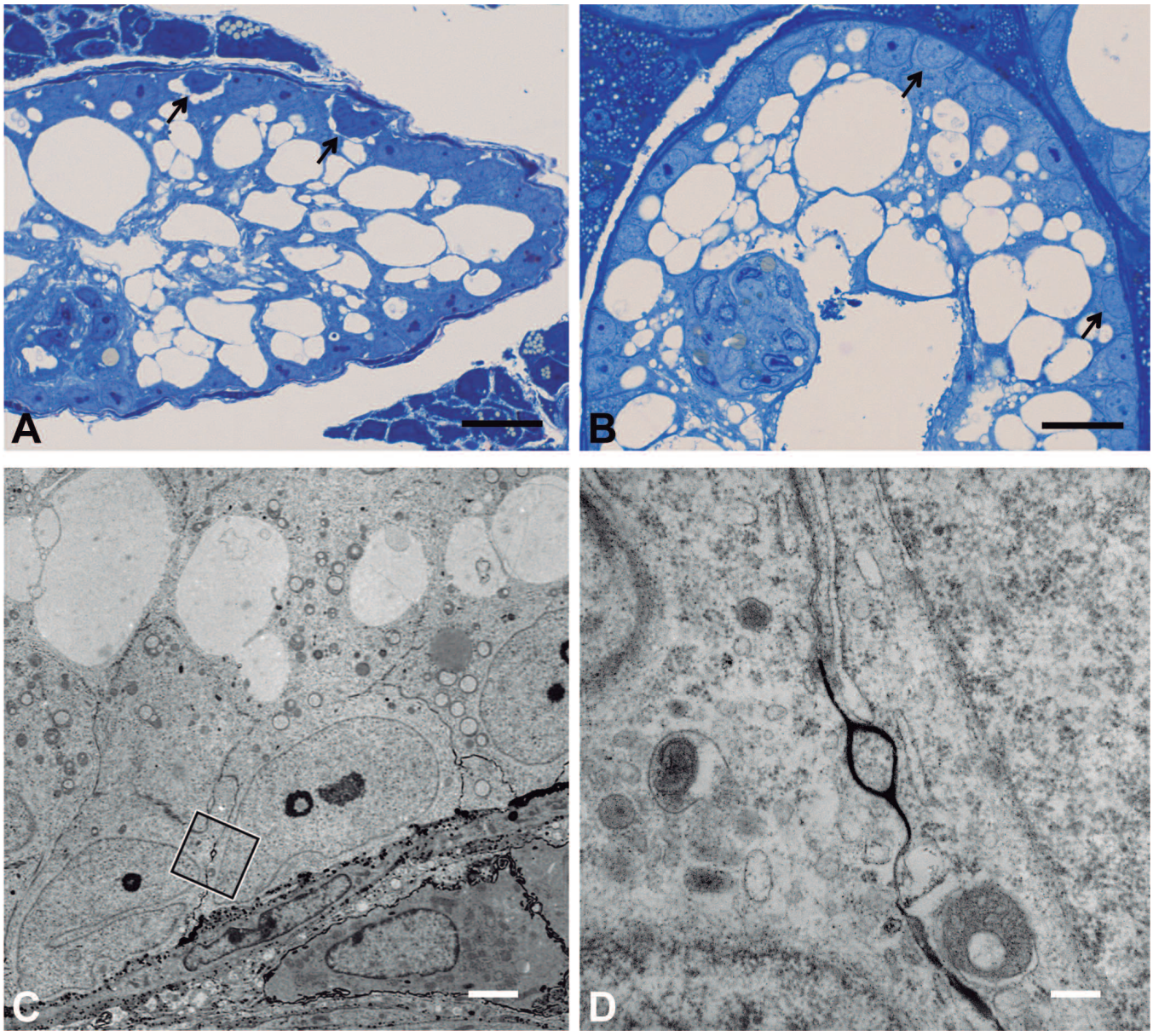

Fig. 4. Functional analysis of BTB integrity in testes of adult SCCx43KO$^{-/-}$ mice at semi- (**A, B)** and ultra-thin **(C, D)** section level (Carette et al., 2010, modified). Perfusion with a hypertonic fixation solution that contains 10% glucose leads to shrinkage artifacts only in spermatogonia (**A,** arrows), indicating an effective BTB. In testes of control KO mice (fixation solution without glucose) no spermatogonia shrinkage artifacts (**B**, arrows) can be observed. KO testes, perfused with a fixation solution containing 2 % lanthanum nitrate, reveal that this electron-dense tracer was stopped by functional inter-SC TJs (**C, D**). **D** enlargement of inset in **C.** Scale bars: A-C, 40 μm; D, 2 μm.

Table 2. Direct evidence for regulatory roles of Cx43 in testicular junction dynamics.

	Consequences on testicular junction dynamics	References
Pan-Cx inhibitory peptides	Reduced expression of occludin and ZO-1 and dyslocalization of N-cadherin	Lee et al., 2006
Anti-Cx43 and anti-plakophilin-2 siRNA	Perturbed inter-SC TJ barrier function; mislocalization of the occludin/ZO-1 complex from the cell surface into the cytoplasm	Li et al., 2009b
SCCx43KO	Increased protein levels of N-cadherin, ß-catenin and occludin but decreased ZO-1 levels; functional intact BTB but enhanced number of TJs and AJs	Carette et al., 2010
anti-Cx43 siRNA and unspecific GJ blockers	Increased protein levels of N-cadherin, ß-catenin and occludin but unaltered ZO-1 levels	Carette et al., 2010

cytoskeleton partners and corresponding signaling pathways. In several epithelia such an inter-junction cross-talk has been demonstrated in recent years (for review, see Derangeon et al., 2009). For example, the interrelated nature of GJs and AJs was already shown by Meyer et al. (1992) in Novikoff cells using antibodies against either Cx43 or N-cadherin, which prevented both GJ and AJ formation. In human fetal astrocytes a reciprocal regulation of Cx43 and TJ protein claudin-1 by interleukin-1, treatment has also been shown (Duffy et al., 2000). Convincing evidence is now accumulating that also in testes Cx43 could influence the expression and function of other junction types (Table 2).

Direct evidence for a possible relationship between Cx43, junction dynamics and spermatogenesis derives from Carette et al. (2010). This most recent study demonstrated that Cx43 based GJs participate in the regulation of AJ and TJ protein expression by using SCCx43KO$^{-/-}$ mice (Fig. 3), specific anti-Cx43 siRNA and GJ blockers in the SerW3 SC line. Interestingly, increased protein levels of N-cadherin, ß-catenin and occludin but decreased ZO-1 levels were observed in adult KO mice compared to their WT littermates. Both anti-Cx43 siRNA and unspecific GJ blockers led to similar results, although ZO-1 levels were unaltered. Using hypertonic glucose and lanthanum nitrate perfusion it has additionally been shown that the integrity of the BTB is still present in adult SCCx43KO$^{-/-}$ mice (Fig. 4). Furthermore, these mutants revealed an increased number of TJs and AJs at the BTB site. Taken together, observed alterations possibly represent an impairment in the dynamic process of opening and closing of this barrier and/or a sign for a permanent BTB closure resulting in impaired spermatogenesis. The inter-relationship between Cx43 and other junctional proteins is also supported by a previous in vivo study using the pan-Cx peptide model (Lee et al., 2006). Intratesticular injection of inhibitory peptides led to reduced expression of occludin and ZO-1 and dys-localization of N-cadherin. The discrepancy between the results of the latter two studies might be explained by the unspecific Cx inhibitory effect of pan-Cxs in all testicular cells compared to specific deletion of the Cx43 gene only in SCs. Additionally, the brief time period during which Cx43 is altered in the pan-Cx model, and the fact that only the cells near the injection site might be affected, could also explain these different results, since in the SCCx43KO$^{-/-}$ mice all SCs are affected throughout life (Carette et al., 2010). A further recent in vitro study showed that Cx43 structurally interacts with the desmosomal protein plakophilin-2 at the BTB site and that the simultaneous knockdown of both Cx43 and plakophilin-2 by RNAi technique can transiently perturb the inter-SC TJ barrier function. This perturbation was accompanied by a mislocalization of the occludin/ZO-1 complex from the cell surface into the cytoplasm, indicating that Cx43 participates in the control of BTB dynamics (Li et al., 2009b). Finally, using calcium switch and bisphenol A models, Li et al. (2010) were able to additionally demonstrate that Cx43 seems to be crucial for TJ reassembly at the BTB during its cyclic restructuring, allowing the transit of preleptotene spermatocytes to the adluminal compartment.

Concluding remarks and future perspectives

Cx43 is the predominant GJ protein in many vertebrate tissues, including the testis. In this review we summarized the recent progress on testicular Cx43 research with respect to its regulatory roles in testicular junction dynamics and spermatogenesis. For example, SC specific KO of the Cx43 gene clearly evidenced that Cx43 represents an absolute requirement for normal spermatogenesis (Brehm et al., 2007; Sridharan et al., 2007). Respectively, loss of Cx43 only in SCs is sufficient to prevent the initiation and maintenance of spermatogenesis. SCCx43KO$^{-/-}$ mice and several *in vitro* studies (Gilleron et al., 2006, 2009) further revealed that Cx43 plays a pivotal role in the negative control of SC proliferation. However, the accurate mechanisms by which Cx43 regulates the development of GCs are still unknown. One possibility is that Cx43 controls the spermatogenic process through regulation of TJs and AJs via peripheral adaptors, cytoskeleton partners and signaling pathways. Direct evidence for such a relationship between Cx43 and junction dynamics derives from Carette et al. (2010), as this study demonstrated that Cx43 based GJs participate in the regulation of AJ and TJ protein expression by using SCCx43KO$^{-/-}$ mice, specific anti-Cx43 siRNA and GJ blockers in the SerW3 SC line. The results also suggest

that Cx43 is most probably involved in the control of BTB dynamics rather than in the maintenance of the barrier's integrity.

Current in vitro studies are dealing with the identification of small signaling molecules, which are involved in BTB formation. SCs derived from WT, heterozygous and homozygous SCCx43KO mice will be compared to develop a mechanistic hypothesis for the regulatory role of Cx43 in spermatogenesis and testicular junction dynamics.

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