A seamless trespass: germ cell migration across the seminiferous epithelium during spermatogenesis

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During spermatogenesis, preleptotene spermatocytes traverse the blood–testis barrier (BTB) in the seminiferous epithelium, which is reminiscent of viral pathogens breaking through the tight junctions of host epithelial cells. The process also closely resembles the migration of leukocytes across endothelial tight junctions to reach inflammation sites. Cell adhesion molecules of the immunoglobulin superfamily (e.g., JAM/CAR/nectin) participate in germ cell migration by conferring transient adhesion between Sertoli and germ cells through homophilic and heterophilic interactions. The same molecules also comprise the junctional complexes at the BTB. Interestingly, JAM/CAR/nectin molecules mediate virus uptake and leukocyte transmigration in strikingly similar manners. It is likely that the strategy used by viruses and leukocytes to break through junctional barriers is used by germ cells to open up the inter–Sertoli cell junctions. In associating these diverse cellular events, we highlight the “guiding” role of JAM/CAR/nectin molecules for germ cell passage. Knowledge on viral invasion and leukocyte transmigration has also shed insights into germ cell movement during spermatogenesis.

Introduction

Cell adhesion molecules of the Ig superfamily (IgSF) typically have one or more Ig-like domains in their extracellular N-terminal region that are implicated in molecular recognition and one cytoplasmic C-terminal region that functions in signal transduction pathways. The Ig-like modules near the N terminus can form linear rods when arrayed in series and are sufficient for homophilic and heterophilic binding (Brummendorf and Lemmon, 2001). They mediate cis-interactions in the plane of the membrane and trans–interaction on opposing cell membranes. In addition, these proteins share common intracellular binding partners, which enables cross talk with other cell surface molecules. These features make IgSF proteins ideal components of cell–cell junctions and cell surface receptors.

Cell adhesion molecules of the Ig family are highly conserved proteins. As they structurally resemble molecules of the adaptive immune system (e.g., antibodies and T cell receptors), genes that encode junctional adhesion molecules (JAMs), cortical thymocyte marker of *Xenopus* (CTX), and nectins were considered to be “fossil” genes that later gave rise to essential elements of the adaptive immune system (Du Pasquier et al., 2004). Indeed, members of the JAM, CTX, and nectin subgroups are expressed on circulating lymphocytes and leukocytes (Ozaki et al., 2000; Moog-Lutz et al., 2003). In vertebrates, they have the propensity to serve as virus receptors at endothelial or epithelial barriers. Their function at the cell junctional complex is not simply that of gatekeepers. They can also transduce signals at the cell membrane, maintain cell polarity, and mediate cell migration. JAM, CTX, and nectin molecules are engaged in a wide spectrum of cellular events ranging from viral infections and leukocyte transmigration to spermatogenesis. They are also essential to the central nervous system (Brummendorf and Rathjen, 1996). Here, we give a full account of the multifaceted characteristics of JAM/CTX and nectin family molecules. By integrating these facts with some of our recent findings, we bring forth a novel understanding of germ cell migration across the seminiferous epithelium during spermatogenesis, which can be tested in future experiments.

General features of JAM/CTX and nectins

The nectin family consists of nectin-1, -2, -3, and -4. They each have three Ig-like domains in the extracellular region and a short cytoplasmic tail. Nectin-like molecules are structurally similar to nectins, but unlike nectins, they do not bind to the peripheral adaptor afadin. Detailed structural and biochemical properties of nectins have been reviewed recently (Ogita and Takai, 2006). CTX family proteins share V- and C2-type Ig-like folds in their extracellular region, highly conserved C-terminal cytoplasmic tails, an extra pair of cysteines, and an N-glycosylation site within their C2 domain (Chrétien et al., 1998). CTX members include coxsackie and adenovirus receptor (CAR), CAR-like membrane protein, the A33 antigen, endothelial cell–selective adhesion molecule (ESAM), and brain- and testis-specific IgSF. Although JAM proteins are a subgroup of the CTX family, JAM-A, -B, and -C are more related to each other in their polypeptide

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Abbreviations used in this paper: BTB, blood–testis barrier; CAR, coxsackie and adenovirus receptor; CTX, cortical thymocyte marker of *Xenopus*; ESAM, endothelial cell–selective adhesion molecule; HSV, herpes simplex virus; IgSF, Ig superfamily; JAM, junctional adhesion molecule; PVR, poliovirus receptor.
sequences than to ESAM, CAR, or JAM-4 (Ebnet et al., 2004). The cytoplasmic tails of JAM-A, -B, and -C are substantially shorter (40–50 amino acid residues only) than those of CAR, ESAM, or JAM-4 (>100 residues). The structures of JAMs and CAR have been comprehensively reviewed elsewhere (Coyne and Bergelson, 2005; Mandell and Parkos, 2005).

### JAM/CTX/nectin molecules as viral receptors

Cell adhesion molecules of the Ig family localize at the subapical surface of polarized epithelial cells. Surprisingly, almost all members of the JAM/CTX and nectin family mediate viral entry and spread (Table I). JAM-A is a receptor for reoviruses (Barton et al., 2001); mammalian reoviruses of serotype 1, 2, and 3 and their respective field strains all bind to JAM-A (Campbell et al., 2005). Viral interaction with JAM-A triggers NF-κB activation and cell apoptosis and may be a defense mechanism or an innate immune response before the start of viral replication (Du Pasquier, 2004; Du Pasquier et al., 2004). JAM-A also has been identified as a receptor for feline calicivirus (Makino et al., 2006).

Nectins also serve as viral receptors and have been reviewed recently (Geraghty et al., 1998; Sakisaka and Takai, 2004; Ogita and Takai, 2006). Both nectin-1 and nectin-2 were originally isolated as poliovirus receptor (PVR)-related proteins, PRR-1 and PRR-2, respectively (Eberle et al., 1995; Lopez et al., 1995). Later they were shown to be receptors for α-herpes virus rather than poliovirus and, hence, were renamed HveC and HveB (Geraghty et al., 1998). Human nectin-like molecule-5 (hNecl-5) is known as the PVR (Mendelsohn et al., 1989; Koike et al., 1990) and mediates entry of porcine pseudorabies virus as well as bovine herpesvirus 1 (Geraghty et al., 1998). Nectin-1 mediates herpes simplex virus (HSV) infection in a wide range of cell types, including fibroblasts, primary sensory neurons, and trabecular meshwork cells of the human eyes (Simpson et al., 2005; Tiwari et al., 2005). This explains the pathogenicity of HSV-1 in these tissues. Access to nectin-1 at the apical surface of polarized cells contributed substantially to HSV infection in vitro (Galen et al., 2006). The clustering of nectin-1 on the membrane protrusions of CHO cells facilitates HSV-1 attachment and a subsequent phagocytosis-like virus uptake (Clement et al., 2006).

CAR is best known for its role as a virus receptor (Bergelson et al., 1997). As a member of the CTX protein family (Chrétien et al., 1998), CAR mediates viral attachment and spread for coxsackie virus group B and adenovirus groups 2 and 5. The availability of CAR on the cell surface is a determining factor for susceptibility to adenoviral gene delivery (Li et al., 1999). However, gene delivery to differentiated epithelia is largely unsuccessful because CAR is sequestered in the intercellular junctional complexes, which transiently disrupts junctional integrity. Note that nectin-1 (PVR) is localized to the basolateral region rather than the apical region of the epithelial cells [Ogita et al., 2001]. Necl-5 forms cis-homodimers but does not mediate trans-homophilic interactions (Aoki et al., 1997).

How viruses reach receptors that are located inside intercellular junctions is an intriguing question that several studies have sought to address. Studies confirming JAM-A as a reovirus receptor were conducted on cell cultures without tight junction structures (Barton et al., 2001). It remains unclear how viruses gain entry to JAM-A in the subapical regions of tight junctions in vivo (Tyler et al., 2001). Nectin-1, when confined to adherens junctions, is not easily accessible to virus either. Release of nectin-1 to the apical cell surface can greatly enhance cell susceptibility to HSV infection (Yoon and Spear, 2002). A novel strategy has been proposed to explain coxsackie virus invasion in the absence of cell surface receptor CAR (Coyne and Bergelson, 2006). To initiate an infection, group B coxsackie virus first interacts with a secondary receptor, decay-accelerating factors, on the cell surface. This in turn activates nonreceptor protein tyrosine kinases of the Abl and Src families, including Fyn. Abl then triggers Rac-dependent actin rearrangement and opens up tight junctions. Once the coxsackie virus reaches tight junctions, viral fiber knobs interact with CAR, replacing the original CAR–CAR homodimers. Viral particles are subsequently internalized by host cells via the caveolin pathway. It is possible that reoviruses, adenoviruses, and α-herpes viruses all conspire to use a secondary receptor on the cell surface to sneak into tight junctions. Interestingly, for almost all viral pathogens, the interfaces of IgSF molecules for binding to viral surface

| Name     | Nomenclature as viral receptors | Mediate viral entry of corresponding viruses |
|----------|--------------------------------|--------------------------------------------|
| JAM-A    | N/A                            | Reovirus and feline calicivirus            |
| CAR      | Coxsackie and adenovirus receptor | Coxsackie B virus and adenoviruses 2 and 5 |
| Nectin-1 | PRR-1 and HveC                 | HSV1, HSV2, PRV, and BHV-1                 |
| Nectin-2 | PRR-2 and HveB                 | HSV2 and PRV                               |
| Nectin-3 | PRR-3                          | N/A                                        |
| Necl-5   | PVR                            | PV, PRV, and BHV-1                         |

See text for references. N/A, not applicable; PRR, PVR-related protein; HveB/C, α-herpes virus receptor; PRV, pseudorabies virus; BHV, bovine herpes virus; PV, poliovirus.

Figure 1. Reovirus, adenovirus, coxsackie virus, HSV, and poliovirus interact with cell adhesion molecules of the IgSF at intercellular junctions. JAM/CAR/nectins not only mediate viral entry into cells but also facilitate newly replicated viral particles to escape from the basal lateral to apical surface for further infection. Viral fiber knobs or other surface proteins bind to JAM/CAR/nectins at the same interfaces that were used in forming homodimers at intercellular junctional complexes, which transiently disrupts junctional integrity. Note that nectin-1 (PVR) is localized to the basolateral region rather than the apical region of the epithelial cells [Ogita et al., 2001]. Necl-5 forms cis-homodimers but does not mediate trans-homophilic interactions (Aoki et al., 1997).
proteins overlap extensively with the domains that mediate homophilic trans-interaction inside junctional complexes (Cocchi et al., 1998; Bewley et al., 1999; Forrest et al., 2003; Guglielmi et al., 2007). It is believed that viral ligands perturb intercellular junctional complexes partly by competing with the homophilic interaction of cell adhesion molecules (Fig. 1).

**JAM/CTX/nectin and cell migration**

Accumulating evidence has pointed to the importance of IgSF cell adhesion molecules in mediating cell migration. They are present not only on endothelial and epithelial cells that are forming junctional barriers but also on circulating leukocytes and platelets. For example, JAM-A and -C proteins are expressed by platelets, neutrophils, monocytes, and lymphocytes (Ozaki et al., 2000; Santos et al., 2002; Nourshargh et al., 2006). JAM-like protein was also detected on human leukocytes (Moog-Lutz et al., 2003).

Several functional studies support the role of JAM proteins in mediating leukocyte transmigration across endothelial tight junctions. Upon treatment with TNFα or IFNγ, JAM-A molecules found within cell junctions are redistributed onto the luminal endothelial cell surface (Ozaki et al., 1999). Leukocytes adhere to the endothelial cells via the interaction between integrins (e.g., integrin αLβ2) on the leukocyte surface and JAM-A on the endothelial cell surface (Oстерmann et al., 2002). Although JAM-B is primarily restricted to endothelial cells, it is involved in trans-heterophilic interaction with JAM-C on leukocytes ( Lamaragn et al., 2005b). Endothelial JAM-C is a counter-receptor for leukocyte integrin Mac-1 (Santos et al., 2002; Chavakis et al., 2004), and overexpression of JAM-C in transgenic mice enhanced leukocyte recruitment to sites of infection (Aurrand-Lions et al., 2005). In fact, JAM-C is the first tight junction molecule reported to promote endothelial permeability (Orlova et al., 2006). In knockout studies, ESAM was shown to support neutrophil extravasation by destabilizing tight junctions via Rho GTPase (Wegmann et al., 2006). JAM-like proteins on neutrophils and CAR inside epithelial tight junctions were also identified as ligand–receptor pairs that mediate neutrophil transepithelial movements ( Zen et al., 2005). CAR is also expressed by primary human endothelial cells derived from pancreatic islets and umbilical veins (Carson et al., 1999; Zanone et al., 2007). Both nectin-2 and nect-5(PVR) are also found at endothelial cell junctions. Nect-5(PVR) forms cis-homodimers but does not mediate trans-endothelial migration. It is noted that as of this writing, homophilic JAM–JAM interaction has not been directly demonstrated for leukocyte–endothelial interactions but remains an attractive possibility. As shown in the drawing, nect-5(PVR) forms cis-homodimers but does not mediate trans-homophilic interactions. CAR-JAM-L interaction is reported on neutrophil transepithelial migration rather than transendothelial migration. We included the CAR-JAM-L complex in the same figure because of its relevance to the subject of leukocyte transmigration.

In culture, cell movement and proliferation are inhibited when two or more cells come into contact with each other and establish cell–cell junctions ( Abercrombie and Heaysman, 1953), a phenomenon known as “contact inhibition.” Nectins are implicated in the establishment of contact inhibition. To initiate junctional formation, necl-5(PVR) trans-interacts with nectin-3 at the colliding edges of two approaching cells ( Ikeda et al., 2004). This trans-interaction activates Cdc42/Rac, which, in turn, triggers actin remodeling to promote junctional formation ( Sato et al., 2005). However, the trans-interaction of necl-5 (PVR) with nectin-3 is transient. Necl-5(PVR) is internalized by endocytosis from the cell surface once cell–cell junctions are established, causing reduction of cell movement and proliferation. Hence, down-regulation of necl-5 has been proposed to be one of the mechanisms underlying contact inhibition ( Fujito et al., 2005). This finding is further supported by increased necl-5 expression during loss of contact inhibition in oncogene-transformed NIH3T3 cell lines ( Minami et al., 2007).

The migratory behavior of cancer cells has been associated with loss of CAR expression in several tissues and cell lines ( Li et al., 1999; Pearson et al., 1999). CAR overexpression considerably reduced cell migration in cervical and ovarian cancer cell lines ( Bruning and Runnebaum, 2004). It also inhibited glioma cell invasion and tumor growth in vivo ( Huang et al., 2005). The cytoplasmic domain of CAR binds tubulin and microtubules, which possibly decreases cell motility through microtubule stabilization ( Fok et al., 2007). JAM-C, on the contrary, was shown to enhance both the adhesion and invasion properties of cancer cells ( Fuse et al., 2007). The N-terminal Ig-like domains of JAM-C mediate trans-homophilic adhesion between tumor and endothelial cells ( Santos et al., 2005). Functional disruption of JAM-C and ESAM could inhibit pathological angiogenesis for tumor growth ( Ishida et al., 2003; Lamagna et al., 2005a).

A mutation in the cytoplasmic tail of JAM-C also abolishes cell polarity and stimulates β1 or β3 integrin–mediated cell migration ( Mandicourt et al., 2007), which converts cells from a static polarized state to a promigratory phenotype.

**JAM/CTX/nectin and spermatogenesis**

In adult mammalian testes, the seminiferous epithelium is composed of Sertoli and germ cells. Sertoli cells create a unique...
environment that provides structural support and nutrients for postmeiotic germ cell development. Inter–Sertoli cell tight junctions compose the blood–testis barrier (BTB), which divides the seminiferous epithelium into two compartments: the spermatagonia-containing basal compartment and the immune-privileged adluminal compartment (Russell, 1977). Spermatagonia differentiate into preleptotene spermatocytes, which are the germ cells that translocate from the basal to the luminal compartment for maturation without compromising the integrity of the BTB (Russell, 1977). This requires rapid disassembly of junctional complexes ahead of migrating preleptotene spermatocytes and instant assembly of these complexes behind moving spermatocytes (for reviews see Russell and Peterson, 1985; Pelletier and Byers, 1992; Mruk and Cheng, 2004). After traversing the BTB, germ cells rely on a series of transient junctions for anchorage onto Sertoli cells during their movement along the seminiferous epithelium.

Cell adhesion molecules of the JAM/CTX and nectin family are abundantly expressed in the testis. Similar to their roles in leukocyte transmigration and viral invasion, JAM/CTX and nectin family molecules participate in germ cell migration through homophilic and heterophilic interactions. Nectin-2 is expressed on both Sertoli and germ cells, whereas nectin-3 expression is strictly limited to spermatids (Ozaki-Kuroda et al., 2002). Nectin-2 also localizes at the inter–Sertoli cell junctions of the BTB. The “ectoplasmic specialization,” a testis-specific junctional structure formed by Sertoli cells, contains F-actin bundles that are arranged at regular intervals beneath the plasma membrane and a cistern of the endoplasmic reticulum connected to microtubules (Cheng and Mruk, 2002). At the Sertoli cell–spermatid interface, nectin-2 and -3 form trans-heterotypic junctional complexes. The nectin-based adhesive membrane microdomains exhibit one-to-one linkage with each F-actin bundle underlying Sertoli cell–spermatid junctions (Ozaki-Kuroda et al., 2002). In the absence of nectin-2 or -3, the ectoplasmic specialization at Sertoli cell–spermatid junctions does not form properly (Mueller et al., 2003; Inagaki et al., 2006). Nectin-2−/− and nectin-3−/− mice both exhibited defective sperm morphogenesis and male infertility (Bouchard et al., 2000; Inagaki et al., 2006). Interestingly, nectin-3 was found on spermatids attached to Sertoli cells but not on spermatooza released from the seminiferous epithelium (Guttman et al., 2004). This may imply that nectin-3 is required to confer the adhesion to germ cells that is necessary for migration across the seminiferous epithelium.

Heterophilic binding of nectin-5(PVR) and necl-2 was recently identified in the interaction between mouse germ and Sertoli cells (Wakayama et al., 2007). Necl-2 is strongly expressed on the germ cell surface but not on Sertoli cells (Wakayama et al., 2003), whereas necl-5 is only present on the Sertoli cells, as demonstrated by electron microscopy. In the seminiferous tubules of necl-2–deficient mice, round and elongating spermatids with a distorted shape failed to attach to the Sertoli cells and were sloughed off into the tubule lumen, resulting in male infertility (Fujita et al., 2006; Surace et al., 2006; van der Weyden et al., 2006; Yamada et al., 2006). Wakayama et al. (2007) first detected the interaction between necl-5 and necl-2 in immunoprecipitation experiments. They then used a culture system to demonstrate that overexpression of necl-5(PVR) in the Sertoli cell line (TM4) increased its capacity to adhere to Tera-2 cells expressing necl-2. This heterotypic interaction between necl-5(PVR) and necl-2 at the Sertoli–germ cell interface may partially explain the indispensable role of necl-2 in spermatogenesis.

JAM-B and -C interact with each other in a manner strikingly similar to the nectin-2–nectin-3 or the necl-5(PVR)–necl-2 complex, with JAM-B coming from the Sertoli cell side and JAM-C from the spermatid side to form heterotypic interactions
CAR was recently identified on the spermatozoa of rats, mice, and humans, where it was observed to occupy the acrosome membrane region (Mirza et al., 2006). Our group reported that CAR is concentrated at inter–Sertoli cell junctions in vitro and the BTB in vivo. Through immunofluorescent staining of isolated germ cells, we observed the presence of CAR on spermatogonia, spermatocytes, and round and elongate spermatids (Wang et al., 2007). Considering that spermatogonia are non-polarized stem cells without acrosome structures, we favor the notion that CAR is present on the germ cell plasma membrane. The presence of CAR on both Sertoli and germ cells suggests that trans-homophilic CAR–CAR interactions might take place. Like JAMs and nectins, CAR can form homodimers, which are mediated by the D1 domain of their two Ig-like loops (van Raaij et al., 2000). During viral infection, viral fiber knobs competitively inhibit CAR–CAR interactions, which either perturb the cell junction mechanically or trigger a signaling cascade to disintegrate the entire cell junctional complex (Walters et al., 2002). In a similar manner, CAR–CAR interactions between Sertoli and germ cells may compete with the original CAR–CAR trans-homodimers between Sertoli cells, thus allowing the passage of germ cells (Fig. 3).

The migration of germ cells across the seminiferous epithelium is highly reminiscent of leukocytes squeezing through tightly apposed endothelial cells. It also bears a close resemblance to viruses traversing adjacent epithelial cells during infections. In all these scenarios, moving cells or viruses first have to break through the tight junction barriers with minimal disruption. The integrity of epithelial or endothelial barriers (e.g., the vascular endothelium with tight junctions, the BTB, or the epithelia of the pathogen host) must not be compromised. After the opening of tight junctions, the progression of these moving cells and viruses relies solely on a series of transient adherens junctions. In addition, these events engage inflammatory cytokines (e.g., TNFα) as common regulators of junctional dynamics. Our group has reported that TNFα is capable of perturbing Sertoli cell tight junction barrier assembly dose dependently in vitro (Siu et al., 2003). TNFα administration to adult rat testes also reversibly perturbed the BTB, making it leaky to a small fluorescent probe such as FITC (Li et al., 2006). Localized production of TNFα from Sertoli and germ cells into the microenvironment at the basal compartment of seminiferous tubules may induce a transient “opening” of inter–Sertoli cell junctions (Li et al., 2006). It is attractive to speculate that strategies used by viruses and leukocytes are also used by developing germ cells to break through tight junctions. When germ cells move along Sertoli cells, the original JAM/CAR/nectin trans-homodimers between Sertoli cells could be replaced by the Sertoli–germ cell junctions composed of CAR–CAR/nectin–2–nectin–3/necl–5–necl–2/JAM–B–JAM–C complexes. Notably, the affinity of trans-homodimers of nectin-3 with nectin-2 was higher than that of trans-homodimers of nectin-2 (Satoh-Horikawa et al., 2000). Likewise, the affinity of JAM-C–JAM-B heterodimers is higher than that of JAM-C–JAM-C homodimers (Lamagna et al., 2005b). As JAM-C can be coimmunoprecipitated with CAR in mouse testis lysates (Mirza et al., 2006), it is intriguing to speculate whether JAM-C on the germ cell surface can form a heterophilic complex with CAR on the Sertoli cell in vivo.

Clearly, JAM/CTX and nectins are just a subset of the junctional molecules that participate in the cell adhesion events that are required for junction formation and stability in the seminiferous epithelium. It is impossible to break through the BTB or other junctional barriers without unlocking occludin- and claudin-based tight junction protein complexes. It is likely that JAM/CTX and nectins are perturbed by heterophilic or homophilic interactions with viral fiber knobs or with the cell surface molecules of leukocytes and germ cells. Disruption of JAM/CTX and nectins may then induce signals via Rho GTPase, Rac-1, or Cdc42 that lead to the breakdown of the entire junctional complex at cell–cell contacts, thereby allowing the passage of migrating cells at the epithelial and endothelial barriers.

Concluding remarks

An increasing body of evidence leads to the perception that transmembrane proteins of the IgSF are not merely passive gatekeepers at cell junctions. These molecules also act to loosen the junctional barrier and guide the passage of migrating cells. For example, endothelial cells create a tunnel lined with platelet/endothelial cell adhesion molecule 1 (PECAM-1) and CD99 to allow leukocyte passage (Muller et al., 1993; Lou et al., 2007). Homophilic engagement of leukocyte PECAM-1 with PECAM-1 expressed on endothelial cells is essential for leukocyte transmigrations. Both JAM-C and ESAM within endothelial junctions were shown to increase endothelial permeability and promote neutrophil extravasation (Orlova et al., 2006; Wegmann et al., 2006). During viral infections, viral fiber knobs bind to JAM-A/CAR/nectin-1 at the same interface that was used for the formation of trans-homodimers inside junctional complexes. Analogously, formation of Sertoli–spermatid junctions is probably achieved through competitive inhibition of existent homodimers between Sertoli cells. By observing together these otherwise unrelated processes, we now look at JAM/CAR/nectin molecules as ushers dispatched by Sertoli cells to guide the passage of germ cells. Unlike leukocytes, germ cells are not actively migrating cells and are not “squeezable.” They rely on the locomotive apparatus in Sertoli cells for support and protrusive force. Hence, understanding the “guiding” role of these molecules at inter–Sertoli cell junctions is of even greater importance. Fortunately, the knowledge that has accumulated on viral invasion and leukocyte transmigration has also shed light on the physiology of germ cell migration across the seminiferous epithelium during spermatogenesis.

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