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Quality control in the epididymis
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·*Review*·

Extracellular quality control in the epididymis

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Abstract

The epididymal lumen represents a unique extracellular environment because of the active sperm maturation process that takes place within its confines. Although much focus has been placed on the interaction of epididymal secretory proteins with spermatozoa in the lumen, very little is known regarding how the complex epididymal milieu as a whole is maintained, including mechanisms to prevent or control proteins that may not stay in their native folded state following secretion. Because some misfolded proteins can form cytotoxic aggregate structures known as amyloid, it is likely that control/surveillance mechanisms exist within the epididymis to protect against this process and allow sperm maturation to occur. To study protein aggregation and to identify extracellular quality control mechanisms in the epididymis, we used the cystatin family of cysteine protease inhibitors, including cystatin-related epididymal spermatogenic and cystatin C as molecular models because both proteins have inherent properties to aggregate and form amyloid. In this chapter, we present a brief summary of protein aggregation by the amyloid pathway based on what is known from other organ systems and describe quality control mechanisms that exist intracellularly to control protein misfolding and aggregation. We then present a summary of our studies of cystatin-related epididymal spermatogenic (CRES) oligomerization within the epididymal lumen, including studies suggesting that transglutaminase cross-linking may be one mechanism of extracellular quality control within the epididymis. *(Asian J Androl 2007 July; 9: 500–507)*

Keywords: epididymis; protein aggregation; extracellular quality control; cystatin; transglutaminase

1 Introduction

The tubular lumen of the epididymis is a unique extracellular environment distinct from many organ systems by the active cell biological process that occurs within its luminal compartment. Within the epididymal lumen spermatozoa acquire the functional capacities of motility and fertility as they migrate from the proximal to the distal region of the tubule. This process is dependent on not only the interaction of sperm with proteins synthesized and secreted by the epithelium but also on the proper maintenance of the epididymal luminal milieu as a whole. Much emphasis has been placed on understanding the role of particular epididymal secretory proteins in the sperm maturation process ultimately to provide new therapies for infertile couples as well as to identify targets for the development of male contraceptives. Less is known, however, regarding the molecular mechanisms that contribute to the overall maintenance of the luminal compartment, including mechanisms to remove secretory proteins selectively as well as those to manage potential problems that can arise from proteins that do not maintain their native structures following secretion into the extracellular space. Because the maturation of spermatozoa is critical for perpetuation of the species, it is likely that surveillance/quality control mechanisms are in place in the epididymal lumen to protect against potential cytotoxicity of protein misfolding and aggregation and to allow maturation to occur. However, to date, the mechanisms of protein aggregation, biological significance, and mechanisms for control/removal of protein aggregates in the epididymis are not known. Understanding the

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mechanisms of extracellular quality control in the epididymal lumen can have far-reaching implications not only for identifying conditions that could contribute to infertility but also will provide new therapies for diseases of extracellular protein aggregation, including Alzheimer's disease, type II diabetes and Creutzfeldt-Jakob disease.

Our laboratory studies the cystatin family of cysteine protease inhibitors, including the members cystatin C and cystatin-related epididymal spermatogenic (CRES) protein and their roles in epididymal and sperm function. On the basis of their inherent propensity to aggregate, we initiated studies to examine protein aggregation in the epididymal lumen using CRES and cystatin C as molecular models. In this chapter, we will first present a background of what is known from other organ systems regarding quality control mechanisms for misfolded and aggregated proteins, mechanisms of protein aggregation with an emphasis on the amyloidogenic pathway, and a brief review of our molecular models. We will then present a summary of our studies of CRES oligomerization in the epididymis, including studies that might identify a mechanism for extracellular quality control.

2 Intracellular and extracellular quality control

Correct protein folding is essential for normal biological function of all organisms. Genetic mutations that can result in a destabilized protein or environmental conditions that promote protein misfolding can lead to cytotoxic protein aggregates associated with numerous disease states, including Alzheimer's, Parkinson's, Huntington's, Creutzfeldt-Jakob disease, type 2 diabetes and cerebral amyloid angiopathy. Prokaryotic and eukaryotic cells use post-translational quality control systems to repair or remove the misfolded proteins. Indeed, the fate of misfolded intracellular proteins is well-studied and includes rescue by chaperones, destruction by proteases, and aggregation. More specifically, chaperones, such as heat shock proteins Hsp90, Hsp70 and Hsp60 are thought to facilitate the correct folding of non-native proteins [1], whereas other chaperones, such as Hsp25 and α-crystallin, bind to exposed hydrophobic regions on unfolded proteins and prevent aggregation until other chaperones can bind [2, 3]. Degradation of misfolded intracellular proteins primarily occurs in the cytosolic proteosome, which degrades proteins that are tagged by ubiquitin in the ER and cytosol. The ER does not, however, catch all misfolded proteins because very recent reports describe the secretion of misfolded transthyretin, suggesting that protein energetics, rather than strict quality control, dictates extent of export from the ER [4]. Once proteins are secreted, they enter the extracellular space in which mechanisms of folding control have yet to be discovered in any organ system. Several studies support the view that extracellular quality control mechanisms exist, including studies showing that denatured plasma proteins are catabolized *in vivo* more rapidly than native counterparts [3, 5]. Although mechanisms similar to intracellular quality control are possible, to date intracellular chaperones have been detected only at low levels in extracellular spaces [3]. Current evidence also does not support a major role for the ubiquitin/proteosome pathway in extracellular quality control, although it is of interest that ubiquitin is present in the epididymal lumen associated with epididymosomes [6, 7]. In general, exposed hydrophobicity in proteins is thought to be the structural change that signals quality control intervention. Studies of extracellular chaperones clusterin and haptoglobin indicate that they recognize hydrophobicity and form noncovalent complexes with non-native proteins and prevent their aggregation, suggesting that these molecules might function as part of an extracellular quality control mechanism [3, 8]. Furthermore, there is a precedent that by forming a complex with non-native proteins, extracellular chaperones can mediate the uptake of their ligands via cell-surface receptors and direct them for intracellular degradation [3]. If indeed extracellular quality control mechanisms exist, then it is likely that a dysfunction of this system could lead to diseases of misfolded proteins. Therefore, defining the mechanisms of extracellular quality control in the epididymis is of high significance not only for reproductive function and treatment of infertility but also could have broad implications for diseases of extracellular protein misfolding, such as Alzheimer's disease, type II diabetes and cerebral angiopathies.

3 Amyloid-type protein aggregation

Proteins can aggregate by several distinct pathways. Nonspecific interactions between proteins in many different conformations can result in the formation of amorphous aggregate structures. Aggregation by the amyloid pathway involves the specific interaction of proteins with a defined intermediate structure ultimately resulting in a higher ordered fibril. The abnormal accumulation of aggregated protein generated by the amyloid pathway is a common feature of degenerative diseases. Aggregated amyloid-β protein is implicated in Alzheimer's disease, whereas an accumulation of aggregated α-synuclein is associated with Parkinson's disease. Cystatin C forms amyloid in the cerebral arteries of patients with amyloid angiopathy. In addition to neurodegenerative diseases, the presence of amyloid

in the testis and epididymis has been implicated in human infertility [9, 10]. In most of these diseases, the end-stage product that accumulates is amyloid fibrils. These structures have been defined by their ability to bind dyes such as Congo red and thioflavin, morphologically as 6–10 nm filaments, and structurally as "cross β" fibrils by X-ray diffraction. It is of interest that amyloid with these characteristic structures can form from unrelated proteins, suggesting that they share a common structural motif and aggregation pathway.

Proteins that have inherent properties for amyloid formation are characterized by a high degree of incompletely solvated backbone H bonds (not protected from water attack), making them vulnerable and, therefore sites of structural weakness [11, 12]. Proteins that fall into this category include known disease-associated proteins, such as amyloid β, α-synuclein, insulin, cystatins and prion proteins [11, 12]. Once destabilized, the protein will self-assemble into higher order structures, including noncovalent dimers, trimers, tetramers, and oligomers that can be up to, or greater than, 1 000 000 daltons. Based on techniques including negative stain electron microscopy and atomic force microscopy, studies of amyloidogenic proteins indicate a common pathway for aggregation. The earliest structures identified by these techniques are spherical particles (3–25 nm in diameter) known as soluble oligomers, which then form a beaded appearance known as a protofibril and, finally, the structures anneal to form mature amyloid fibrils [13]. These mature amyloid fibrils may then continue to grow by the addition of destabilized monomers. The recent development of conformation-dependent antibodies that specifically recognize early-stage [14] and late-stage soluble oligomers [15], and fibrillar forms [16] but not monomeric forms or proteins in native conformations has provided valuable reagents for study of these structures independent of peptide sequence.

Although previously the amyloid fibrils were thought to be the causative form in disease, a large number of studies now indicate that the soluble oligomeric or protofibrillar forms are cytotoxic and cause the disease [13]. Because unrelated amyloidogenic proteins as well as proteins not associated with disease can adopt similar cytotoxic structures, one line of thought is that protein structure rather than sequence is responsible for the cellular toxicity [17]. Alternatively, a disruption of the equilibrium between monomeric and oligomeric forms of a protein might cause pathologies because of decreased monomer or new functions of the aggregated protein. Nevertheless, it appears that protein aggregation is more widespread than previously thought and that organisms have developed mechanisms to protect against amyloidogenesis. Currently, studies in the amyloid field are focused on identifying the mechanisms of soluble oligomer-induced cellular toxicity and evidence suggests a disruption of the cell membrane, possibly by the formation of permeable channels, and subsequent calcium dysregulation and apoptosis leading to cell death [18, 19].

4 Transglutaminases (TGase)

Transglutaminases (TGases) are a family of calcium-dependent enzymes that catalyze γ-glutamyl-amine cross-links between reactive glutamine and lysine residues resulting in a covalent bond resistant to denaturation. TGase-mediated isopeptide bonds can form between two distinct proteins with one protein contributing the glutamine and the other the lysine, or result in the cross-linking of amines, such as polyamines or the fluorescent tag monodansylcadaverine, to proteins with reactive glutamine residues. Complete TGase substrates in which one protein has both the reactive glutamine and lysine residues are rarer but can result in both intramolecular and intermolecular cross-links. Nine TGase genes have been identified, eight of which encode active enzymes. TGase are members of the papain family of cysteine proteases and are highly conserved at their active site. TGase2 or tissue-type TGase (tTGase) is perhaps the most-studied family member and is present both intracellularly and extracellularly in many tissues, including the testis and epididymis. Although tTGase has been implicated in cell proliferation, differentiation and apoptosis, its functions remain enigmatic. It has been proposed that tTGase functions might vary depending on its tissue and cellular localization [20]. In particular, the presence of different accessible proteins to act as tTGase substrates might determine its cell type-dependent activity and function. In support of this, several studies have suggested a role for tTGase in stabilizing protein aggregates, therefore contributing to diseases such as in Alzheimer's disease [21, 22], whereas other reports suggest a protective effect of tTGase by virtue of its forming protein cross-links and, therefore, protein aggregates in a nontoxic conformation distinct from that in a disease-related oligomer [23, 24]. By diverting protein aggregates from the amyloid pathway, nontoxic aggregate structures may then be targeted for endocytosis. This hypothesis is supported by studies showing that TGase is involved in receptor-mediated endocytosis [26]. Although studies proposing a protective effect of tTGase on protein aggregation were carried out by examining intracellular amyloidogenic proteins [25], tTGase is also

present extracellularly and as such may perform similar functions as a means of extracellular quality control, including within the epididymis. Although TGases in general have proposed roles in reproduction, including sperm motility [27] and formation of the seminal coagulum [28], tTGase knockout mice are viable and fertile [29, 30]. Redundancy among TGase family members is thought to contribute to the lack of a defined phenotype. However, whether the tissue-type TGase activity in the testis and epididymis represents the same tTGase gene product that was knocked out is not known, nor have detailed studies of reproductive function in the tTGase knockout mice been carried out.

5 Epididymal luminal environment

The epididymis consists of a single long convoluted tubule through which spermatozoa must pass following their release from the testis. Testicular spermatozoa are nonfunctional in that they lack progressive motility and the ability to fertilize and only acquire these functions after undergoing sperm maturation in the epididymis. Because sperm are synthetically inactive, the maturation process involves the interaction of sperm with proteins that are synthesized and secreted by the epididymal epithelium. Epididymal protein synthesis and secretion is highly regionalized such that proteins expressed and secreted in one region of the tubule may not be expressed in adjacent regions. In addition to active protein secretion into the epididymal lumen, other proteins are removed. Although the mechanisms of protein removal from the lumen are not well-understood, the epithelium has been shown to carry out fluid phase, adsorptive and receptor-mediated endocytosis [31]. Together, region-dependent protein secretion and reabsorption by the epithelium result in spermatozoa encountering an ever-changing luminal environment as they migrate from the caput to the cauda region, which ultimately results in sperm maturation.

By far the majority of studies in the epididymis have focused on identifying specific epididymal secretory proteins and their functional roles in sperm maturation ultimately to identify novel targets for male contraception or to provide new treatments for male infertility. However, equally important as these individual proteins, is understanding the complex epididymal luminal milieu as a whole because perturbations in the microenvironment that surrounds the sperm cell could affect maturation. The epididymal lumen contains perhaps the most complex fluid found in any exocrine gland resulting from the continuous changes in composition, as previously mentioned, as well as the presence of components in unusual concentrations (for reasons not yet known), or those not present in other body fluids [32]. The caput epididymidis, the region the spermatozoa first enter coming from the testis/efferent ducts, is the most metabolically active region, secreting 70–80% of the total overall protein secretion in the epididymal lumen [32]. Moreover, within this same region, 99% of the fluid accompanying the testicular sperm is resorbed, resulting not only in a profound concentration of spermatozoa but luminal components as well [33]. Although such an environment might be integral for sperm maturation, an environment low in water content creates a situation of macromolecular crowding, which places unique stresses on luminal proteins that can alter their behavior and lead to protein misfolding and aggregation [34, 35]. Other stressors, such as inappropriate ionic strength, oxidative stress, and pH and temperature extremes, are also known to promote the unfolding of fully folded native proteins and the formation of aggregated proteins, which can be cytotoxic. Considering that this same epididymal environment must protect spermatozoa and allow maturation, it is likely that mechanisms are in place to prevent or remove aggregated proteins. However, to date, virtually nothing is known regarding protein aggregation in the epididymal lumen, including mechanism of formation, biological significance, and mechanisms for control and/or removal.

Several recently published reports provide evidence that the epididymal fluid does not just consist of a large pool of soluble proteins in their native conformations, but rather also contains proteinaceous aggregate structures of varying molecular mass. In particular, the amyloidogenic prion protein is present in the epididymal lumen both in insoluble exosome-like membranous vesicles (epididymosomes) [36], and in a soluble high molecular mass lipophilic complex in association with hydrophobic proteins [37]. The chaperone clusterin, which is known to interact with hydrophobic proteins to maintain their solubility, was also detected in the soluble prion protein complex. This suggests that these structures might be a means to maintain proteins in their soluble state either to prevent aggregation and precipitation and to enable clearance or to allow hydrophobic proteins to be transferred between cells, such as the epididymal epithelium and spermatozoa. Although still not well-characterized, the epididymosomes are small membrane-bound vesicles in the lumen that are thought to arise from apocrine type secretion from the epithelium and to be a means to transfer hydrophobic proteins to the maturing sperm cells [38, 39].

Other evidence for the presence of protein aggregates in the epididymal lumen comes from studies examining

molecular chaperones in the reproductive tract. Both heat shock protein 1 (HSPD1, HSP60) and tumor rejection antigen 1 (TRA1, a member of the heat shock protein 90 family) colocalize to large electron dense bodies in the epididymal lumen. These structures seemed not to be membrane bound and are larger than epididymosomes, suggesting unique structures [40]. Because proposed functions of TRA1 include the folding of denatured proteins as well as multimer assembly [41], its function in the epididymal lumen might be as a means of extracellular quality control, specifically to refold proteins in non-native conformations or alternatively to facilitate clearance. Most interesting is that TRA1, as well as other heat shock family members, are substrates of TGase cross-linking [20]. These data support the possibility that TGase might participate in the removal of protein aggregates from the epididymal lumen. Also of interest is that the extracellular chaperone, clusterin, is abundantly secreted in the epididymis of many species and contributes an amazing 41% of the total protein secreted in the rat caput epididymidis, suggesting an important role for this protein in epididymal function, possibly as a mediator of extracellular quality control [32]. Finally, there is tantalizing evidence from other reports describing a dense, filamentous material in the epididymis, which might also represent protein aggregation [42].

Taken together, these studies suggest that protein aggregation is an integral part of normal epididymal function because there appear to be no obvious detrimental effects of these structures on the maturation of spermatozoa. It is likely that in the epididymal lumen there is a delicate equilibrium between proteins in monomeric and aggregate states and that mechanisms for controlling/neutralizing potentially cytotoxic aggregates are in place. Indeed, the presence of chaperone proteins, some of which are transglutaminase substrates, in most of the aggregate structures identified thus far support this interpretation.

6 Cystatins and the L68Q cystatin C mutation

The cystatins are a superfamily of tight binding but reversible cysteine protease inhibitors consisting of three families: stefins, cystatins and kininogens [43]. The family 2 cystatins, represented by cystatin C, are small (14–16 kDa) secretory proteins. Although it is well-established by *in vitro* studies that the cystatins are potent cysteine protease inhibitors, their *in vivo* functions are less clear. Because of its amyloidogenic properties, cystatin C is implicated in several diseases. Notably, cystatin C contributes to protein deposits in cerebral arteries of patients with age-related cerebral amyloid angiopathy, including that associated with Alzheimer's disease [44], whereas in the hereditary form of the disease a point mutation (L68Q) leads to a destabilized and, therefore, highly amyloidogenic form of cystatin C that forms protein deposits in patients who die before age 30 of hemorrhagic stroke [45]. Detailed studies of cystatin C oligomerization showed that it forms dimers by the process of 3-D domain swapping and as such is inactive as a cysteine protease inhibitor [46]. Studies of cystatin C protein aggregation are important for information that can be gained regarding the biological consequences of, or mechanisms by which, cystatin C aggregation may be prevented and would ultimately be beneficial for diseases associated with these processes. Because of its causative role in neurodegenerative diseases, little attention has been paid to its putative role in reproduction and, in fact, little is known regarding cystatin C in the reproductive tract other than localization in Sertoli cells [47], germ cells [47] and the epididymis [48] and that cystatin C knockout mice are fertile [49]. Whether male individuals with the hereditary form of cystatin C angiopathy (L68Q) exhibit subfertility or infertility is not known, likely as a result of the fact that most individuals die early in life and probably before attempting to father children. However, in L68Q patients, cystatin C amyloid has been detected in tissues outside of the brain, including in the testis [50].

7 CRES

CRES is the defining member of a new subgroup within the family 2 cystatins [48, 51]. Although sequence, predicted structure, and chromosomal mapping data indicate that CRES is a secretory cystatin, it is distinct from the typical cystatins by its reproductive-specific expression and lack of conserved sites necessary for cysteine protease inhibition, predicting distinct biochemical activities. Indeed, our *in vitro* studies showed that CRES did not inhibit cysteine proteases but rather inhibited prohormone convertases, specifically PC2, a substrate-specific serine protease that processes precursor hormones/proteins to their mature and active forms [52]. Therefore, one role for CRES might be to mediate proprotein processing events. Within the epididymis, CRES is synthesized and secreted by the proximal caput epididymal epithelium, accumulates in the lumen of the midcaput, but abruptly disappears from the distal caput epididymal lumen [53]. The mechanism(s) for the sudden disappearance of CRES is not known. Because most pro-hormone convertases function within the secretory pathway, it is possible that CRES function might also be intracellular, specifically within the secretory granule. Therefore, its presence in the epididymal lumen might merely be a result of its being part of the secretory gran-

ule contents that are released upon secretion and, as such, may then be targeted for removal.

Throughout our years of study with CRES we observed that it had a high propensity to self-aggregate in all types of expression systems, including bacterial expression, secretion from *Pichia pastoris*, and in *in vitro* transcription and translation. Using the PoPMuSiC algorithm that predicts protein instabilities and likelihood to self-aggregate [54], CRES showed sites of incompletely solvated H bonds (i.e. structural weakness that were located in similar positions to that in cystatin C). That crystallography showed these regions in cystatin C to be sites of local unfolding, and subsequent refolding in a dimeric state supports the view that CRES might self-aggregate by a similar mechanism [46]. Furthermore, cystatin C dimer formation was shown to be the first step in the pathway towards amyloid formation [55], also implying CRES potential for amyloidogenesis.

8 CRES oligmerization in the epididymal lumen

Studies were initiated to determine whether CRES forms oligomeric structures *in vivo* on the basis of two observations. First, we were intrigued by the abrupt and complete disappearance of CRES from the caput epididymal lumen [53]. Although we can not rule out that other mechanisms such as proteolytic degradation or endocytosis are involved, the lack of data supporting these processes as the sole means of CRES disappearance prompted us to consider other possible mechanisms. Because cystatin C, as well as several other cystatins, had been shown to self-aggregate we questioned whether CRES could also self-aggregate and considered that perhaps our inability to detect CRES in the lumen of regions downstream of the midcaput could be due to its presence in high molecular mass oligomeric structures. Using size exclusion chromatography to fractionate caput luminal fluid followed by Western blot analysis, we determined that a proportion of CRES was detected in the void volume, suggesting its presence in high molecular mass structures. The use of the homo-bifunctional sulfhydryl reversible chemical cross-linker DSP allowed us to trap CRES heterodimeric and oligomeric structures that were reversed to the 19 and 14 kDa CRES monomers in the presence of reducing agents. These studies suggested that the high molecular mass structures represent, in part, self-aggregates of CRES. Further studies of caput fluid using size exclusion chromatography and SDS-agarose gels allowed us to detect high molecular mass CRES oligomeric structures that were resistant to SDS. Taken together, there studies revealed that CRES was present in the caput epididymidal lumen in multiple forms, including soluble SDS-resistant and SDS-sensitive oligomeric structures, as well as in monomeric forms. Size exclusion chromatography and Western blot analysis of luminal fluid from all regions of the epididymis showed the presence of SDS-resistant CRES oligomeric structures in the proximal caput region, an accumulation in the midcaput, and a continued presence in the distal caput region. Monomeric CRES, however, was only detected in the proximal and mid-caput regions. This suggests that the "disappearance" of CRES from the epididymal lumen might indeed reflect its association with high molecular mass structures, which might be less antigenic and difficult to detect by immunohistochemistry and not resolved on standard SDS-PAGE gels used in our initial studies to examine CRES protein.

9 Mechanism of CRES protein aggregation

Studies were next carried out to determine the molecular mechanism of CRES protein aggregation. Because cystatin C is known to form amyloid in several disease conditions, we determined whether CRES might aggregate by the amyloidogenic pathway. Recombinant CRES protein incubated for extended times at 37ºC was examined by negative stain electron microscopy. These studies showed that over time CRES formed soluble oligomers and protofibrils typical of amyloidogenic proteins. Furthermore, after several days of incubation CRES formed fibrils that stained with Congo red, a conformation-dependent dye that is used clinically to diagnose amyloid. These studies demonstrate that *in vitro* CRES aggregates by the amyloidogenic pathway and studies are in progress to determine whether CRES aggregates *in vivo* are also by this process.

10 CRES is a substrate for TGase

Because a proportion of oligomeric CRES in the caput fluid was present in SDS-resistant high molecular mass structures, we next examined the potential mechanism for formation of the stable oligomeric structures. TGase are known to form stable cross-links that are resistant to denaturation between glutamine and lysine residues. Therefore, experiments were carried out to determine whether TGase cross-linking could be involved in the formation of stable CRES oligomers. Incubation of recombinant CRES protein with guinea pig liver TGase, calcium and the fluorescent amine monodansylcadaverine (MDC) resulted in a time-dependent fluorescent labeling of CRES protein that was inhibited by the presence of EDTA and TGase inhibitors. These studies indicated that CRES had reactive glutamine sites that were substrates for TGase cross-linking. Ad-

ditional studies in which CRES was incubated with TGase but without MDC demonstrated a time-dependent disappearance of the CRES monomer that was prevented by the addition of TGase inhibitors. These studies suggest that CRES is a complete TGase substrate and contains both reactive glutamine and lysine residues that are substrates for TGase and that following exposure to TGase CRES will form high molecular mass oligomeric structures that do not enter a typical SDS-PAGE gel. Additional studies including 14C-putrescine incorporation assays showed the presence of TGase activity within the epididymal lumen with highest activities in the proximal caput region and that endogenous CRES is a substrate for both exogenous and endogenous TGase cross-linking and will form SDS-stable high molecular mass oligomers in the caput fluid. To determine whether TGase cross-linking of CRES altered its conformation, CRES was incubated with guinea pig liver TGase and examined by negative stain electron microscopy. These experiments showed that TGase cross-linking caused CRES to form an amorphous protein aggregate distinct from the highly organized oligomeric structures of the amyloidogenic pathway. Therefore, TGase cross-linking might divert CRES from the potentially cytotoxic amyloidogenic pathway and instead form aggregates that are nontoxic.

11 Summary

We propose that the formation of protein aggregates is a natural process occurring within the epididymal lumen. Because these protein structures can be cytotoxic either by a mechanical disruption and/or properties inherent in their oligomeric structures, the epididymis utilizes extracellular quality control mechanisms to protect sperm maturation and epididymal cell function. Although extracellular control mechanisms have not yet been described in any organ system, the presence of significant amounts of transglutaminase activity and extremely high levels of clusterin in the epididymal fluid, suggest that protein cross-linking and chaperones might be functioning either independently or synergistically to control protein aggregation in the lumen. The epididymal tubule and its luminal compartment are like no other extracellular environment in the body in that sperm maturation must occur despite a surrounding environment that favors protein misfolding and aggregation. The unusual composition and extraordinary levels of some components in the epididymal fluid might represent an extracellular environment that has gone to extreme measures to control problems/toxicities that are known to exist with aggregate structures. It is also quite possible that once formed oligomeric structures carry out biological functions within the lumen.

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