

# Clusterin in the male reproductive system: localization and possible function

Robert Bailey \*, Michael D. Griswold

*Department of Biochemistry and Biophysics Washington State University, Pullman, WA 99164-4660, USA*

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## Abstract

Clusterin is a glycoprotein that was initially isolated from the male reproductive system. Subsequently, clusterin has been found to be widely distributed in a variety of tissues in mammals. One characteristic of the expression of clusterin is that it is induced as a result of cellular injury, death, or pathology. Despite the efforts of many laboratories working in diverse biological systems, the function of clusterin remains unknown. Recent studies have revealed a 'heat-shock element' in the promoter of the gene that may account for the inducible nature of the clusterin gene. Overall, the evidence suggests that function of clusterin is to protect surviving cells after damage. This protection may result from a detergent-like action of the protein. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Clusterin; Male reproductive system; Cellular injury

## 1. Introduction

Clusterin is a ubiquitously expressed amphipathic glycoprotein consisting of two non-identical subunits designated  $\alpha$  and  $\beta$ . The 34 kDa  $\alpha$  and 47 kDa  $\beta$  subunits of rat clusterin are held together by disulfide bonds (Kissinger et al., 1982). Early in the study of clusterin, many investigators from multiple disciplines were pursuing apparently non-related proteins. When the cDNA sequence of rat Sertoli cell clusterin, also known as sulfated glycoprotein-2 (SGP-2) was reported, numerous proteins from various tissues and species were found to be clusterin homologues (Collard and Griswold, 1987). As a result, clusterin is known by several other names including sulfated glycoprotein-2, glycoprotein III, testosterone repressed message-2, GP-80, SP-40, 40, cytolysis inhibitor, and apoJ (see historical perspective in Fritz, 1995). One of the first studies of the protein by Fritz and colleagues used ram rete testis fluid as a source. The isolated protein was capable of

causing aggregation of various cells in vitro so the protein was designated 'clusterin,' and most investigators in the field have agreed to use this designation (Blaschuk et al., 1983a,b). The function of clusterin is not well defined but it has been implicated in a variety of physiological roles. Some putative functions include; cell-cell interactions (Fritz et al., 1983), sperm maturation (Sylvester et al., 1991), inhibition of complement attack (Jenne and Tschopp, 1989), lipid transport (de Silva et al., 1990), and membrane remodeling during apoptosis (Tenniswood et al., 1992).

## 2. Localization of clusterin in the male reproductive tract

The earliest studies of clusterin were done in the reproductive system where clusterin was identified as a major protein in the secretory products of cultured rat Sertoli cells and the fluid of ram rete testis (Kissinger et al., 1982; Blaschuk et al., 1983a,b). In the rat testis, clusterin is synthesized by Sertoli cells, secreted into the

\* Corresponding author.

lumen of the seminiferous tubules, and is the dominant protein in the spent medium of cultured rat Sertoli cells (Griswold et al., 1988). Rat seminiferous tubule fluid and rete testis fluid contain 50–60 µg/ml clusterin (Sylvester et al., 1991). Immunohistochemistry in rat testis sections using clusterin antibodies revealed staining of the cytoplasm of most Sertoli cells and intense staining of the heads and tails of late spermatids and released spermatozoa (Sylvester et al., 1991). Washed testicular sperm exhibited staining over the acrosome, the neck and along the tail. At the ultrastructural level, immunogold particles bound to clusterin antibody were found associated with lysosomes and residual bodies in Sertoli cells, with the outer membrane of spermatid tails and with the membrane of the sperm head. Detergents were required to remove clusterin from testicular sperm, indicating that the protein was tightly associated with the membrane (Sylvester et al., 1984, 1991).

Northern analysis and *in situ* hybridization showed that mRNA for clusterin was found in highest abundance in the epididymal cells (Sylvester et al., 1991). An interesting and important phenomenon was also observed in the reproductive tract of the rat: a higher molecular weight testicular form of clusterin ( $\alpha = 37$  kDa;  $\beta = 42$  kDa) was present on testicular sperm but a lower molecular weight epididymal form was present on caput sperm ( $\alpha = 29$  kDa;  $\beta = 37$  kDa). The differences in these molecular weights were due exclusively to differences in the oligosaccharide moieties (Mattmueller and Hinton, 1991; Sylvester et al., 1991). The testicular form of clusterin is endocytosed by cells in the rete testis and efferent duct epithelia, and the immunodetectable clusterin was found to be absent in the efferent ducts (Sylvester et al., 1991; Veeramachaneni and Amann, 1991). Immunogold localization of clusterin in the rete testis and efferent duct epithelia showed that the protein associates with endocytic vesicles (Herms et al., 1991). A model has been proposed whereby testicular clusterin is removed from sperm during transit from the testis and the epididymal form of the protein is reapplied to outer sperm membranes in the caput epididymis (Sylvester et al., 1991). Endocytosis of clusterin may be facilitated by glycoprotein 330/megalyn also known as LRP-2, a cell surface receptor expressed on the epithelial cells lining the efferent ducts proximal caput. LRP-2 and clusterin colocalize to the coated pits and endocytic vesicles in the efferent ducts (Morales et al., 1996). These findings underscore some important facts: the testicular form of clusterin must have a local function within the seminiferous tubules, and the presence of clusterin is required throughout the reproductive tract.

Clusterin is also localized to the prostate. Tenniswood and co-workers found that after castration of rats, expression of clusterin or testosterone-repressed prostate message-2 (TRPM-2) increased in the ventral

prostate (Léger et al., 1987) in relation to cellular damage (see below). Clusterin has been localized to the cytoplasm of epithelial cells in the proximal region of the prostatic duct (Sensibar et al., 1991). Also, the prostate form of clusterin is glycosylated differently than testicular and epididymal clusterin (Sensibar et al., 1993).

Although prominent in the entire reproductive tract of the male, less is known about where clusterin is expressed in the female reproductive tract. Northern analysis showed that clusterin mRNA is expressed in the human ovary and the surface epithelia of the uterus and the uterine glands (Sylvester and Griswold, 1995).

### 3. Structure and regulation of the clusterin gene

Clusterin appears to be the translation product of a single gene in several mammalian species (Jenne and Tschopp, 1989; Slawin et al., 1990). The complete genomic organization and sequences of the rat, human, and mouse clusterin gene and the promoter region have been published (Wong et al., 1993, 1994; Jordan-Starck et al., 1994). The clusterin gene is organized into nine exons and eight introns and is approximately 14 kb in the rat and 17 kb in humans. There is a high degree of conservation between species in the amino acid and nucleotide sequences of the protein and the gene.

Clusterin is ubiquitously expressed and its expression is tightly regulated. The level of its expression is normally low but it increases tremendously by environmental and pathophysiological stress (reviewed in Rosenberg and Silkens, 1995). The expression of clusterin in Sertoli cells in testis is different. It is constitutively expressed at such a high level that it comprises up to 70% of the total mass of proteins secreted by Sertoli cells in primary cultures, while its up-regulation by different stimuli is relatively weak (Griswold et al., 1988; Clark and Griswold, 1997).

Several recent reports address the transcriptional control of the clusterin induction. In mouse, the involution of the mammary gland may stimulate clusterin expression by means of the nuclear factor 1 (Furlong et al., 1996). In the mink lung epithelial cell line CCL64, transforming growth factor  $\beta$  may induce clusterin expression through the action of the transcription factor API (Jin and Howe, 1997). Finally, heat-shock induction of clusterin in HeLa, A431, human epithelial carcinoma, and quail QT6 cell lines may be mediated by the heat-shock transcription factor 1 (Michel et al., 1997). The heat-shock element was found to be absolutely conserved in clusterin promoters in different classes of vertebrates and was proposed to mediate the stress inducibility of clusterin in general.

#### 4. Structure of clusterin protein

The mature protein has an acidic pI and is comprised of two non-identical disulfide linked  $\alpha$  and  $\beta$  subunits with extensive charge heterogeneity. In the rat, clusterin is synthesized as a single chain precursor containing a signal peptide of 21 amino acids that is removed in the mature protein (Collard and Griswold, 1987). There are two clusters of five cysteine residues in clusterin, and all are involved in interchain disulfide bonds and appear to be conserved among species (Choi-Miura et al., 1992). Before secretion, the single chain is glycosylated and cleaved forming the disulfide linked  $\alpha$  and  $\beta$  subunits (Fig. 1). There are six *N*-linked glycosylation sites in rat clusterin. In some tissues, the oligosaccharides are sulfated resulting in a highly negatively charged product with amphipathic helices (Griswold et al., 1986). The carbohydrate moiety has been extensively analyzed in human clusterin, and was approximately 17–27% of the molecular weight as determined by mass spectral analysis, and contained a heterogeneous mixture of carbohydrates, the most abundant being of the bisialobiantennary variety (Kapron et al., 1997). Interestingly, human serum clusterin is not sulfated as is the rat Sertoli cell clusterin. The relevance of these differences is unknown at this time, but the pattern of glycosylation is probably tissue specific and plays a role in protein function (Tenniswood et al., 1998).

Some insight into possible functions of clusterin has come from detailed computer analyses of the protein sequence. The amino acid sequence of clusterin contains four domains of potential amphipathic helices, domains which show a distant relation to myosin tail, and a consensus sequence for a dinucleotide binding

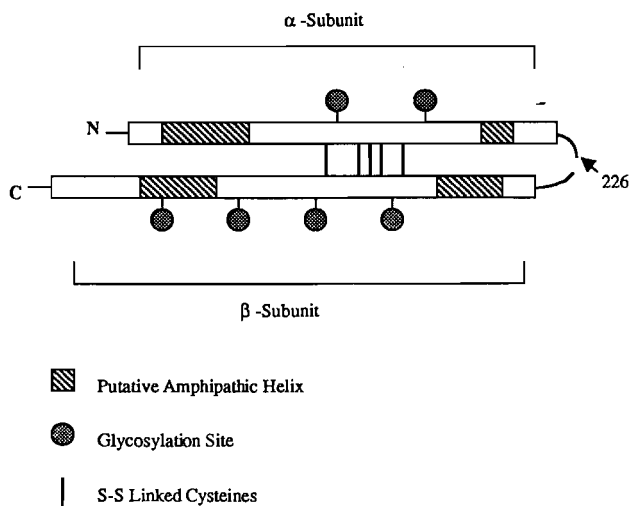


Fig. 1. Schematic structure of rat clusterin. Six *N*-linked glycosylation sites are distributed unevenly between two disulfide-linked subunits. Cleavage of the single chain precursor into the  $\alpha$  and  $\beta$  subunits occurs at residue 226. Each subunit contains two putative amphipathic helical regions.

site, and four heparin binding sites (de Silva et al., 1990; Tsuruta et al., 1990a,b). Clusterin has also been predicted to have the potential to form coiled-coil structures (Jenne and Tschopp, 1989). The amphipathic  $\alpha$ -helical structures would allow for interaction of clusterin with hydrophobic molecules (Tsuruta et al., 1990a,b).

#### 5. Induction in cellular damage and heat-shock conditions

One common theme found in several tissues is that an increase in clusterin expression occurred as a result of cell injury or during programmed cell death. Many investigators working with different tissues have shown that clusterin is induced at the mRNA and protein levels in regressing, involuting or injured tissues, such as testosterone-deprived prostate (Léger et al., 1987), kidney disease and ablation (Bettuzzi et al., 1991; Eddy and Fritz, 1991; Harding et al., 1991; Correa-Rotter et al., 1998), and neurodegeneration (see May and Finch, 1992; May, 1993; May et al., 1996).

In order to examine the induction of clusterin as a result of damage in the testis, adult rats were treated with methoxyacetic acid (MAA), an alkylating agent that selectively destroys pachytene spermatocytes through apoptosis within three days of a single bolus application (Clark et al., 1997). At intervals up to 72 h post-treatment, immunohistochemical labeling of clusterin in testes from control animals was localized to Sertoli cell cytoplasm, the surface of late elongated spermatids, and the cytoplasm of relatively few earlier germ cells. At 6 h after MAA treatment, however, clusterin protein was also localized to the cytoplasm of pachytene spermatocytes. This staining occurred before any morphological changes in the spermatocytes were seen. Pyknosis of the degenerating spermatocytes was apparent by 12 h after MAA treatment, and the cytoplasm of these cells still stained for clusterin at this and later time points. Complete degeneration of the spermatocytes had occurred by 72 h after treatment with MAA. We also determined that Sertoli cells secreted the clusterin protein that accumulated in the spermatocyte cytoplasm. It is unknown how the clusterin protein was taken into the degenerating germ cells. Also, accumulation of clusterin protein in pachytene spermatocytes of MAA-treated rats was seen before any noticeable morphological degeneration of the germ cells or apoptotic fragmentation of their DNA had occurred. Because of clusterin's known transport of, and binding to, hydrophobic macromolecules in other tissues, it is possible that in the MAA-treated testis, Sertoli cell-derived clusterin could have accumulated in pre-apoptotic spermatocytes to isolate potentially harmful cellular components away from viable germ cells. In the

normal, untreated testis, this proposed mechanism could be involved in the clearance of normally degenerative germ cells or transport of hydrophobic components for remodeling of germ cell membranes during the process of spermatogenesis.

We also characterized the heat-stress-induced increase in the expression of testicular clusterin in both the mouse Sertoli cell line MSC1 and rat Sertoli cells (Clark and Griswold, 1997). In order to determine if clusterin expression under these conditions was regulated in a testis-specific manner, the responses of testicular cells were compared to that of non-testicular cells (A431 cells). The mRNA for heat-shock protein 70 (HSP70) was measured to compare the expression of this stress-regulated gene with that of clusterin.

Rat Sertoli cells, MSC1 cells, and A431 cells were cultured under heat stress conditions (41°C) for up to 48 h and the steady-state amounts of mRNA for clusterin and HSP70 were examined by Northern blot analysis. The up-regulation of clusterin mRNA in A431 cells was similar to that of HSP70, suggesting that expression of clusterin may be regulated in a manner similar to that of heat-shock proteins in this cell type. In contrast, there was a delayed increase in clusterin mRNA in heat stressed MSC1 and Sertoli cells that may indicate a Sertoli-specific regulation of the expression of clusterin. There was a relatively low level of induction in cultured primary Sertoli cells, that is likely due to the high basal level of expression in these cells. The act of putting them in tissue culture is probably an inductive process.

## 6. Binding of clusterin to biological molecules

In recent years, clusterin has been shown to bind with high affinity to several different biological molecules. Clusterin binds with high affinity to glycoprotein 330/megalin, also known as LRP-2, which is a member of the low-density lipoprotein receptor family (Kounnas et al., 1995). The binding could be competitively inhibited with excess clusterin as well as other LRP-2 ligands such as apo E, lipoprotein lipase, and lipoprotein-related protein or LRP. In cell culture, only cells that expressed LRP-2 could bind and internalize labeled clusterin (Hammad et al., 1997). The authors speculated that LRP-2 is a receptor for clusterin. This idea gained support from the studies of Morales and co-workers that show a high level of LRP-2 in the efferent ducts where the testicular form of clusterin is removed from the tubular fluid (Morales et al., 1996).

Clusterin has also been shown to bind very tightly to the amyloid  $\beta$  peptide. Clusterin may act as a carrier of amyloid  $\beta$  and may protect cells against amyloid  $\beta$  toxicity (Oda et al., 1995). Clusterin also

Table 1

Clusterin has been shown to bind with high affinity to all of the following

Paroxynase	Mackness et al. (1997)
Apolipoprotein A1	Jenne et al. (1991)
Cell surface of <i>Staphylococcus aureus</i>	Partridge et al. (1996)
LRP-2 (lipoprotein-related protein) $K_d = 14$ nM	Morales et al. (1996)
5b-9 Complement	McDonald and Nelsestuen (1997)
Type I and II TGF $\beta$ receptor C term 127	Reddy et al. (1996)
Amyloid $\beta$ 1–40 $K_d = 2$ nM	Matsubara et al. (1995, 1996); Oda et al. (1995) and Boggs et al. (1996)
Heparin $K_d = 60$ nM	Pankhurst et al. (1998)

appears to associate with lipids of HDLs in complex with apo A1, and with the membranes of epididymal sperm (James et al., 1991; Law and Griswold, 1994; Oda et al., 1995). Clusterin was also found to bind to heparin which could aid in the localization of clusterin to sites of tissue damage (Pankhurst et al., 1998). Other studies (Table 1) have shown the binding of clusterin to apolipoprotein A (Jenne et al., 1991), paroxynase (Mackness et al., 1997), the surface of *Staphylococcus aureus* (Partridge et al., 1996), complement components (McDonald and Nelsestuen, 1997), and transforming growth factor  $\beta$  (Reddy et al., 1996). We contend that many of these interactions are characteristic of the general hydrophobic binding nature of clusterin.

## 7. Conclusions

Is it possible to derive some basic underlying theme from the diverse results discussed above? Either clusterin is a multi-functional protein, or its appearance in various tissues and its association with various processes has some fundamental link. The following general statements pertain to clusterin from all sources:

(1) Many tissues make a basal level of clusterin. Clusterin is also induced in a wide variety of tissues in response to cellular injury and/or insult. The original findings that point to an association of clusterin with apoptotic cells are another manifestation of this response. It is now very clear that the clusterin is made primarily by the surviving cells or the cells next to the dying cells (French et al., 1994; Clark et al., 1997). Clusterin is induced by heat-shock in many cell types and has been shown to have a heat-shock element conserved in the promoter (Michel et al., 1997). Clusterin can be considered a secreted heat-shock or acute phase protein.

(2) In many tissues including the reproductive system, epithelial cells secrete clusterin into a lumen. Many cell types that secrete clusterin are highly secretory and consist of the cells surrounding tissue-fluid interfaces (Aronow et al., 1993).

(3) The protein has amphipathic properties, i.e. it is soluble and has a pI of about 4.0 and yet it binds tightly to hydrophobic surfaces. Clusterin contains four to five amphipathic helices associated with the distal arms, i.e. located away from the clustered cysteines. These amphipathic regions have a number of important properties including myosin-like rod structure (Tsuruta et al., 1990a,b), possible coiled-coiled structures (Jenne and Tschopp, 1989), and a relative lack of defined structure (unpublished observations).

These general properties lead to the following hypothesis: Clusterin functions as a biological detergent. While this possibility has been generally discussed among investigators in this area, it has most recently been advocated by Witte et al. (1995). Clusterin binds readily to unfolded hydrophobic regions of proteins, to hydrophobic membrane proteins, and to some lipids, where it may function to solubilize these molecular complexes and to prevent their accumulation and the subsequent blockage of the ducts or lumen. Binding of clusterin to hydrophobic molecules and complexes may also promote their clearance by assisting their uptake into cells. Uptake into cells is aided by the association of clusterin with a variety of cell-surface receptors including GP-330 (see Fig. 2). The biological action of clusterin is a function of its ability to bind tightly to a wide variety of hydrophobic molecules. Important structural aspects of clusterin aid this function. The Cys containing domains and the glycosylated regions of the protein may function as a scaffold domain, and the binding activity results from the amphipathic nature of the 4 antiparallel helical domains.

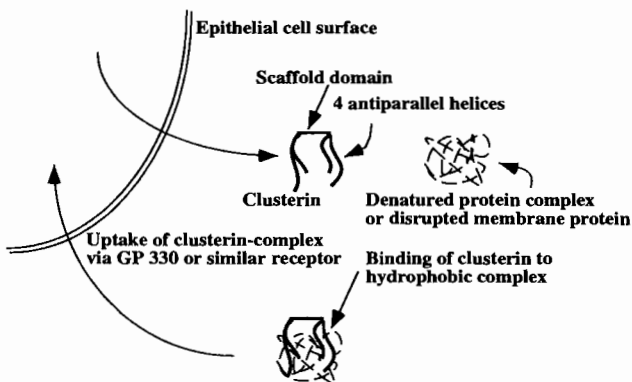


Fig. 2. Simplistic model for clusterin action. Clusterin is shown diagrammatically with a scaffold domain (five disulfide bonds) and four amphipathic helices. We propose that clusterin acts like a detergent, and binds to hydrophobic complexes and denatured proteins, aiding in their uptake or clearance.

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