

## Heat Shock Proteins in Human Endometrium Throughout the Menstrual Cycle

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### ABSTRACT

Human endometrium, in response to steroid hormones, undergoes characteristic cycles of proliferation, secretory changes, and tissue shedding. Human endometrium expresses a molecular repertoire which includes the heat shock proteins (Hsps) Hsp27, Hsp60, Hsp70, Hsp90, and alpha crystallin B chain. The expression of Hsp27, Hsp60, and the constitutive form of Hsp70 (Hsc70) shows a sharp increase in human endometrium after ovulation. The maximal expression of the molecular chaperone, alpha crystallin B chain, occurs during the secretory phase. In view of known functions of the Hsps, it is likely that these proteins are involved in protection of the endometrial proteins against factors with the potential to lead to protein denaturation. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a cytotoxic cytokine that is produced in progressive amounts during the secretory phase. The function of the Hsps may be to protect cells against the cytotoxic damage of TNF- $\alpha$ , particularly during the critical period of "implantation window." *Infect. Dis. Obstet. Gynecol.* 7:5-9, 1999. © 1999 Wiley-Liss, Inc.

### KEY WORDS

endometrium; heat shock protein; alpha crystallin B chain; human; menstrual cycle; stress; uterus; cytokine; tumor necrosis factor

### HISTORY AND DEFINITION

Over 30 years ago, Ritossa et al. noted that an increase in the incubation temperature of *Drosophila melanogaster* larvae results in the development of a defined set of new transcription loci on the polytene chromosome.<sup>1</sup> Twelve years later, the first gene products involved in this response were identified, and the term "heat shock protein" (Hsp) was applied to this family of proteins.<sup>2</sup> Subsequently, it was shown that these proteins exist in all species and are well conserved.<sup>3</sup> Even in thermophilic organisms, a sudden temperature upshift leads to the overexpression of Hsps. However, while the regulation of Hsps was studied extensively, relatively little was known about their physiological significance.<sup>3</sup> The discovery of folding helper proteins

(chaperones) did not contradict Anfinsen's theory, which states that the acquisition of the specific three-dimensional protein structure depends exclusively on the amino acid sequence of the individual protein.<sup>4</sup> Molecular chaperones do not change the protein folding; rather, they maintain proteins in the correct folding pathway and prevent nonspecific interactions.<sup>5</sup> Later, it was found that the expression of Hsps is also increased in response to other environmental factors, and, therefore, they are more appropriately called stress proteins. It has become clear that stress proteins participate in a diverse range of actions in cells. This includes thermotolerance, stress tolerance, cell proliferation, cell metabolism, embryogenesis, and drug resistance. Stress proteins also act as molecular chaperones.

Grant sponsor: Public Health Research; Grant No: CA46866 (S.T.).

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These proteins assist in cellular protein folding and prevent irreversible side-reactions, such as nonspecific aggregation of proteins.

### FUNCTION OF THE HEAT SHOCK PROTEINS

The steroid hormones act by binding to the steroid hormone receptors that regulate transcription of the target genes in steroid hormone responsive tissues. The function of the steroid hormone receptors is regulated by a group of constitutively synthesized Hsps. These factors are expressed at a relatively low level under normal conditions but are inducible by a number of signals, including stress, steroid hormones, and cytokines.<sup>6,7</sup> The Hsp70 family is the most conserved group of proteins within the Hsp superfamily.<sup>8</sup> Other members of this family include the nucleolar Hsp110,<sup>9</sup> the highly conserved Hsp90 family,<sup>10</sup> mitochondrial Hsp60,<sup>11</sup> collagen-binding Hsp47,<sup>12</sup> and a heterogeneous family of small Hsps with molecular weights ranging from 16 to 40 kDa.<sup>3</sup> Of these, in humans, only Hsp27/Hsp28 has been identified thus far.<sup>13</sup> The Hsp60 family consists of proteins that constitutively are highly expressed and are moderately stress inducible. The Hsp70 family is comprised of several proteins that are localized in distinct cellular compartments. The constitutively synthesized protein, designated HSC70, which is found in the cytosol and nuclei of cells, is only moderately stress inducible. On the other hand, under normal conditions, the most strictly inducible member of this family of proteins is not found in the cytosol and nuclei of cells in most species except primates. The Hsp90 family of proteins is abundant and is comprised of constitutively synthesized cytosolic proteins that are only moderately stress-inducible.

The term "molecular chaperone" is applied to proteins that prevent incorrect interactions of proteins and participate in the assembly of proteins without being part of the final protein structure.<sup>14,15</sup> A considerable amount of evidence suggests that members of the Hsp family act as molecular chaperones, e.g., Hsp60 and Hsp70 participate in the folding and unfolding of cellular proteins.<sup>6</sup> Hsp90 interacts with steroid receptors, tubulin, actin, and several protein kinases and prevents the aggregation of citrate synthase and casein kinase II *in vitro*. Nonliganded forms of the steroid

hormone receptors (aporeceptor) exist as complexes associated with various members of the Hsps, such as Hsp90, Hsp70, and Hsp56 (p59), a 40-kDa cyclophilin-related protein, and an uncharacterized 22-kDa protein species.<sup>7,16-18</sup> The alpha crystallin consists of two types of highly homologous 20/22-kDa alphaA and alphaB subunits.<sup>19,20</sup> The A and B chains noncovalently self-associate to form a large macromolecular complex of approximately 40 subunits.<sup>19,20</sup> Although originally it was believed that the expression of the alpha crystallin was strictly confined to the lens, this protein has been found in a variety of normal tissues.<sup>21</sup> More recently, evidence accumulated that allows classifying these proteins as small Hsps<sup>22</sup> and identifies them as molecular chaperones.<sup>23,24</sup>

### EXPRESSION AND POTENTIAL FUNCTIONS OF HEAT SHOCK PROTEINS IN HUMAN ENDOMETRIUM

In human endometrium, the expression of Hsp90 protein showed minimal changes throughout the menstrual cycle. On the other hand, the expression of Hsp27, Hsp60, and HSC70 increased progressively during the late proliferative and early secretory phases and diminished in the mid- to late secretory and menstrual phases.<sup>25</sup> In contrast, the inducible form of Hsp70 did not undergo these changes.<sup>25</sup> With the exception of Hsp70, which was found primarily in the epithelial cells, the immunoreactivity for other Hsps was found in both the stroma and the epithelium. Immunoreactivity for Hsp27 was found in the lymphoid aggregates within endometrial stroma, and both Hsp27 and Hsp90 were found in the endothelial cells. The immunoreactive Hsps were found in the nuclei and/or cytoplasm of cells. However, no consistent nuclear versus cytoplasmic staining emerged, and such localization was irrespective of the site, the cell type, or the phase of the menstrual cycle. Our findings showed that the endometrium has a full complement of Hsps.<sup>25</sup> The expression of Hsp90, Hsp70, and Hsp27 has been reported previously in human endometria.<sup>26,27</sup> Koshiyama et al.<sup>27</sup> reported that both Hsp70 and Hsp90 were expressed in human endometrium throughout the menstrual cycle. The expression of Hsp70 was also found to be stronger in the epithelium than the stroma, whereas Hsp90 was present in both the epithelium and stroma. However, in that report there was no

indication of the intracellular distribution of these proteins. In addition, although the expression of Hsp70 was found in the basalis, it was reported to be weak in the functionalis during the proliferative phase. The expression of Hsp90 was also found to be weak during the secretory phase.<sup>27</sup> The underlying basis for these differences remains to be determined but may be related to differences in the processing of the tissues. In a study by Nip et al.,<sup>26</sup> the two forms of the Hsp could not be resolved by Western blot analysis. In our report,<sup>25</sup> the two forms of Hsp were resolved, and the progressive increase in the relative amount of Hsp70 was more pronounced for the constitutive rather than the inducible form of this protein. The subject population in the study by Nip et al.<sup>26</sup> consisted of women who were either infertile or had uterine bleeding, which did not allow for an evaluation of the Hsps in normal menstrual cycles. However, based on the Western blot analysis, it was suggested that the relative amount of Hsp70 was increased in the infertile group when compared with those with uterine bleeding.<sup>26</sup>

By using representational difference analysis (RDA), we identified the alpha crystallin B protein in human endometrium. This protein was absent in the nonreceptive proliferative endometrium and appeared in the surface epithelium of the human endometrium within the "implantation window."<sup>28</sup> Both the mRNA and protein of alpha crystallin B chain exhibited a similar pattern of expression. The relative abundance of alpha crystallin B chain mRNA and protein progressively increased during the secretory phase.<sup>28</sup> The immunohistochemical staining showed that, in human endometria, this expression was virtually confined to epithelium. The progressive increase of the alpha crystallin B protein in the secretory phase was largely attributable to the increased alpha crystallin B chain in the glandular epithelium.<sup>28</sup> Among the physiological signals, both systemic and local factors may be implicated in the regulation of alpha crystallin B chain in the epithelial cells. Among the steroid hormones, estrogen regulates the production of a small 24-kDa Hsp.<sup>29</sup> Expression of the mRNA of this protein was significantly induced in the MCF7 breast carcinoma cells by estrogen.<sup>29</sup> We tested whether the expression of alpha crystallin B chain is also regulated by steroid hormones.<sup>28</sup> We showed that the expression of alpha crystallin B chain

mRNA is increased by medroxyprogesterone acetate (MPA) and by estrogen withdrawal. However, the same treatments did not change the level of expression of Hsp27 mRNA. Therefore, the progressive rise in the amount of endometrial alpha crystallin B chain during the secretory phase may be attributable to the progressive rise in the systemic level of progesterone followed by the estrogen withdrawal.<sup>28</sup> Progesterone is known to induce 20-dihydroprogesterone dehydrogenase in the endometrial epithelium, the enzyme that inactivates E2 by converting it to E1.<sup>30</sup> Furthermore, progestins downregulate estrogen receptor (ER).<sup>31</sup> These actions of progesterone are, therefore, reminiscent of the E2 withdrawal. A recent report on the alpha crystallin B chain promoter region in humans did not indicate presence of progesterone response elements, however, it showed presence of several cis-acting sequence elements, including multiple half-site estrogen response elements.<sup>32</sup> Such findings suggest that the expression of alpha crystallin B chain mRNA may be directly regulated by E2. Immunocytochemical studies using monoclonal antibodies to ER have shown that estrogen receptor disappears from glandular epithelial cells in the mid-secretory phase of the menstrual cycle.<sup>33</sup> The inhibitory influence of E2 and the stimulatory effects of MPA on alpha crystallin B chain in the *in vivo* experimental system are consistent with these findings. Local factors may also be implicated in the alpha crystallin B chain mRNA expression. For example, it has been shown that, in glial cells, exposure to tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) resulted in the accumulation of mRNA of alpha crystallin B chain.<sup>34</sup> Therefore, it is conceivable that the expression of the alpha crystallin B chain may also be regulated, at the local level, by the endometrial TNF- $\alpha$ .

The function of the Hsps in human endometrium, particularly those of the Hsp 70 family and the alpha crystallin B protein, may be to limit the extent of cytotoxic damage by cytokines or apoptosis. Leukocytes produce high levels of reactive oxygen species as well as cytokines, both with the ability to regulate the expression of Hsps.<sup>35</sup> Since leukocytes accumulate progressively in human endometrium during the secretory phase,<sup>36-38</sup> and the amount of endometrial TNF- $\alpha$  increases progressively during the secretory/menstrual phase,<sup>39,40</sup> the function of Hsps in human endometrium may be to protect

cells from the side effects of leukocyte accumulation or cytokine release. The presence of cytokines such as interleukin (IL)-1 and TNF- $\alpha$  leads to the activation of phospholipase A<sub>2</sub>, the generation of lipid mediators of inflammation, and a rapid rise in the concentration of mitochondrial reactive oxygen radicals.<sup>35</sup>

On the other hand, Hsps intervene with the DNA strand breaks and lipid peroxidation imposed by the reactive oxygen species and protect mitochondrial structure and function.<sup>35</sup> It has been demonstrated that cells transfected with Hsp70 are protected from cytotoxic damage by TNF- $\alpha$ .<sup>41</sup> Furthermore, the overexpression of Hsp70 and not Hsp27, or the inhibition of endogenous Hsp70 synthesis by the expression of antisense Hsp70 RNA, did not change the ability of the cells to bind TNF or to internalize and degrade the receptor-bound ligand. The TNF-induced activation of NF-kappa B-like transcription factors was also unaffected by altered concentrations of Hsp70, suggesting that resistance to TNF- $\alpha$ , conferred on the cells by the overexpression of Hsp70, was not mediated by changes in either the TNF receptors or the regulation of transcription of genes whose expression is regulated by NF-kappa B-like transcription factors. Protection against TNF- $\alpha$  seems to correlate best with the activation of arachidonic acid metabolism.<sup>41</sup> Based on these studies, it has been proposed that resistance against TNF- $\alpha$  endowed by Hsp70 is mediated by the reduced activation of phospholipase A<sub>2</sub>. In addition, some studies show that heat shock or chemical stress, which induce an Hsp response, concomitantly inhibits IL-1 beta and TNF- $\alpha$  production at the transcriptional level.<sup>42</sup> When mouse peritoneal macrophages stimulated with lipopolysaccharide (LPS) were heated at 45C for 12 min, a reciprocal increase in the expression of Hsp70 and a decrease in the production of IL-1 and TNF- $\alpha$  were observed.<sup>43</sup> In line with these findings, heat shock prevented LPS-induced TNF- $\alpha$  synthesis by rat mononuclear phagocytes.<sup>44</sup>

The inference from these and similar studies is that the role of Hsp70 may be to oppose the action of TNF- $\alpha$  in human endometrium and to limit the extent of cytotoxic damage by this cytokine. The number of apoptotic cells is low during the proliferative phase in endometrium. However, the number of these cells increases progressively, particu-

larly in the endometrial epithelium, reaching a peak during the menstrual phase.<sup>39</sup> Since Hsp70 can protect cells from apoptosis,<sup>34,45</sup> and alpha crystallin B chain confers resistance against the cytotoxicity induced both by the TNF- $\alpha$  and oxidative stress, the function of these proteins may be to limit the extent of these events in the endometrium, particularly during the critical period of "endometrial receptivity."

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