Although a wide range of physical and chemical agents disrupt gametogenesis and embryogenesis in animals and humans, much less is known about the mechanisms leading up to these disruptions and the factors that determine whether a particular animal or human will be sensitive to a potential toxicant. Recent research has identified a number of different stress protein families, also known as heat shock proteins (HSPs), that play a role in protecting cells and organs from the toxic effects of exposure to hyperthermia and chemical insult (1). These stress proteins could provide significant insight into cellular processes susceptible to toxicant action and the mechanisms that have evolved to protect them. Understanding these protective mechanisms will allow for more accurate extrapolation of animal data to humans and thereby improve the assessment of human health risk for a potentially broad range of reproductive and developmental toxicants.

To explore the role of stress proteins in reproductive toxicology, the Reproductive Toxicology Division of the National Health and Environmental Effects Research Laboratory of the U.S. Environmental Protection Agency (EPA) held a mini-workshop on 1 November 1996 in Research Triangle Park, North Carolina. Seven intramural and extramural scientists (see shaded box), experts in the field of HSPs, presented findings from their respective laboratories and participated in a roundtable discussion of research issues significant to the workshop theme. The research and its implications for toxicology are summarized here.

This information promises to provide insights into toxicological processes modulated by HSPs. Furthermore, this research will advance the EPA's long-term goal to develop a scientific basis for assessing and characterizing risks to human health.

Mammalian Heat Shock Proteins Relevant to Reproductive Toxicology

Stress proteins are typically categorized by molecular mass into families of homologous genes and proteins (Table 1). In gametes and embryos, exposure to a wide range of toxic agents results in the increased expression and/or subcellular translocation of 27-, 70-, 90-, and 105-kDa HSPs (2–7). This workshop included reports on efforts to characterize the expression and function of stress proteins in rodent gametes and embryos, to create transgenic models wherein the expression of HSPs has been altered, and to use these models to understand biological processes such as cell cycle control and apoptotic cell death, which involve stress proteins and are significant to reproductive toxicology.

**HSP27.** HSP27 is constitutively expressed in most mammalian cells; however, the level of HSP27 expression and phosphorylation status of the protein can be increased by exposure to heat and other toxicants (6). In rat testes, Hsp27 expression varies with the cycle of the seminiferous epithelium, and HSP27 protein is associated with microfilaments in Sertoli cells (7). Hsp27 expression is low in seminiferous tubules at stages that are differentially sensitive to agents that disrupt spermatogenesis (e.g., cadmium), and this sensitivity may be related to the degree of HSP27 stabilization of Sertoli cell microfilaments at different stages of the spermatogenic cycle. The level of HSP27 expression has also been positively correlated with embryonic stem cell vulnerability to heat and metal toxicity (8), and similar correlations between vulnerability to toxicants and HSP27 have also been made in rat embryos exposed to hyperthermia (6). Thus, HSP27 appears to be involved in the development of thermostolerance and sensitivity to heat and chemical exposures in rodent testes and embryos. Whether HSP27 stabilization of microfilaments in Sertoli cells is related to thermostolerance remains to be proven.

**HSP70s.** HSP70s are the most widely studied and well defined of the stress protein families. Multiple Hsp70 genes and encoded proteins have been characterized in mammals (9–11) and the expression of stress-inducible HSP70s is linked with exposure to a range of toxic agents including heat, sodium salicylate, and arsenic (5). Furthermore, in the rat whole-embryo culture model, induction and nuclear translocation of stress-inducible HSP70s positively correlates with both the onset and duration of thermostolerance (12–13). Whether the stress-inducible HSP70-1 and 70-3 are necessary and sufficient to prevent arsenite-induced neural tube defects in mouse embryos has been tested in a series of loss-of-function/gain-of-function experiments (14). Antisense inhibition of HSP70-1 and 70-3 expression resulted in an eightfold-higher incidence of neural tube defects in embryos exposed to normally subteratogenic doses of arsenite. Gain of HSP function was accomplished using a constitutive-promoter transgene construct that overexpressed HSP70-1 and significantly reduced embryo sensitivity to arsenite-induced neural tube defects (14). Thus, both direct and correlative data support the assertion that stress-inducible HSP70s protect embryos from the effects of toxicant exposure.

Besides the stress-inducible HSP70s, a number of different HSP70s are expressed either constitutively or during specific developmental phases in gametes and embryos (15). One example of developmental regulation of a heat shock protein is Hsp70-2 expression in mouse spermatocytes (16). To determine if HSP70-2 has a critical role in meiotic spermatogenesis, the Hsp70-2 gene was disrupted in mice (17). Gene knockout of Hsp70-2 resulted in pachytenic spermatocytes that became apoptotic and arrested at the G2/M-phase transition of meiosis I (18). Furthermore, the cyclin B1-CDC2 complex required for the G2/M-phase transition failed to assemble and become an active kinase in the mutant spermatocytes (19).

Thus, the phenotype of the Hsp70-2 knockout mice indicates a previously unrecognized relationship between an HSP70, meiotic cell cycle, and germ cell apoptosis. The possibility of similar roles for other HSP70s in gametes and embryos is an area of active research because maintenance of the cell cycle and appropriate apoptosis is critical to normal development.

**Other HSPs.** Exposure of gametes and embryos to toxic agents results in the induced expression of a number of other HSPs in addition to HSP27 and HSP70s. In vivo and in vitro exposure of organogenesis-stage rat or mouse embryos to toxic doses of heat or arsenic induces expression not only of...
HSP70s but also HSP90 and HSP105 (3–4, 20). However, the connection between expression of different classes of HSPs in the same reproductive tissues is currently unknown, beyond the interaction of HSP90s with HSP70s during complexation with steroid receptors. The expression of HSP70 partner proteins such as the DNAJ-homologue HSP40 have been linked to the development of thermotolerance in somatic cells (21), and an HSP47 has been identified, which is constitutively expressed and heat-inducible in the developing neural plate of rat embryos (22). Whether this HSP47 is a DNAJ-homologue or whether it interacts with HSP70s (23) remains to be determined.

Future Research

Future research will attempt to address the following questions:

- In what biological processes do HSPs participate and how does this participation translate into protective mechanisms significant to reproductive toxicology? Exposure to toxic agents induces a range of biological effects that include the induction of stress proteins (Fig. 1). Transgenic animal gain-of-function and loss-of-function models should prove useful in testing the relationships between the expression of specific HSPs and other biological effects of exposure, and determining how these biological effects translate into adverse reproductive or developmental outcomes.
- What is the relationship between different families of HSPs in modulating reproductive and developmental toxicity?

Determining whether different HSPs protect the same biological processes and whether HSPs provide additive or synergistic effects will be significant to developing complete models of in vivo systems. Various combinations of HSP transgenic models will be useful in understanding how different types of HSPs interact with each other in gametes and embryos.

- How will understanding HSP function be applied to estimating human health risks? Will it be possible to build quantitative models of toxicant action based upon HSPs as a common pathway? Conducting and assimilating mechanistic research is part of the EPA’s effort to improve animal-to-human extrapolation and build biologically based dose–response models for use in risk assessment (24). Expanding and deepening our understanding of HSP functions will help in developing generalized mathematical models of the progression of toxicity.

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