MONOCLONAL ANTIBODY TO A HEAT SHOCK COGNATE PROTEIN (hsc71) RECOGNIZES RELATED ANTIGENS SYNTHESIZED DURING LATE SPERMATOGENESIS IN THE MOUSE

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Both prokaryotic and eukaryotic cells respond to environmental or physiological stress by synthesizing heat shock proteins. The most prominent is a 70kDa heat shock protein (hsp70), which is highly conserved during evolution. Two hsp70-related heat shock cognate (hsc) proteins of 71kDa (hsc71) and 74kDa (hsc74) are present in mouse cells (11, 13). It has been demonstrated recently that mouse spermatogenic cells synthesize hsp70 in response to heat stress and also constitutively express a cell-specific 70kDa protein (P70) which is similar to hsp70 (1, 2). The functions of the heat shock proteins are still uncertain. Recent studies have shown that hsc71 is identical to the uncoating ATPase which releases clathrin from coated vesicles (4, 16). It has been hypothesized that heat shock proteins, including hsc71, are involved in assembly and disassembly of a variety of protein complexes in vivo (14).

During the preparation of monoclonal antibodies against proteins from mouse spermatogenic cells, we identified an antibody (13D3) which recognizes hsc71. Using this antibody in immunoblotting and indirect immunofluorescence studies, we have found that antibody 13D3 identified other proteins synthesized late in spermatogenesis which appear to be unique heat shock proteins in male germ cells.

I. MONOCLONAL ANTIBODY 13D3 RECOGNIZES hsc71

Monoclonal antibody 13D3 was found fortuitously during immunoblot screening of clones for antibodies to another protein. The antibody reacted strongly with a 71kDa protein on two-dimensional (2D) immunoblots of mouse spermatogenic cells. This protein migrated to the same location on 2D gels as a heat shock protein family member, hsc71, previously identified in
spermatogenic cells (1,2). Further evidence that antibody 13D3 recognized hsc71 was obtained by examining unstressed and heat-stressed 3T3 cells. Hsc71 and hsc74 are present in unstressed 3T3 cells, while hsp70 synthesis is induced by heat stress in these cells. When antibody 13D3 was used on 2D immunoblots of these cells, it was found to bind strongly to hsc71, faintly to hsp70, and not at all to hsc74.

Hsc71 is synthesized during early mouse embryogenesis (3) and in mouse embryonal carcinoma cells (7,12), and hsc71 transcripts have been detected in all mouse cell lines and tissues examined (6,8). Nucleic acid sequence data of hsc71 genes or cDNAs from rat, human and mouse show that this protein is highly conserved in mammalian species (5,6,13,15). One role of hsc71 in mammalian cells involves its ATPase activity (4,15), but it is likely that there are also others. High conservation of hsc71 during evolution and its constitutive presence suggest that it has a fundamental role in cell function. Because antibody 13D3 is more specific for hsc71 than previously reported antibodies, it may prove to be a useful tool for learning additional functions of hsc71.

II. ADDITIONAL PROTEINS APPEAR DURING SPECIFIC STAGES OF SPERMATOGENESIS

Pachytene spermatocytes, round spermatids and condensing spermatids were isolated by unit gravity sedimentation, and proteins from these cells were analyzed by 1D immunoblotting. In populations of pachytene spermatocytes or round spermatids, only one band (hsc71) was seen, whereas, the additional band was detected in condensing spermatids and in cauda sperm extracts. Only one band (hsc71) was detected when proteins from Sertoli cells were examined. These observations were extended by 2D immunoblots. Antibody 13D3 reacted identically with hsc71 on 2D immunoblots of pachytene spermatocytes and round spermatids, but recognized two additional proteins (70kDa/pI 6.4 and 70kDa/pI 6.5) in condensing spermatids and spermatozoa. Another protein (69kDa/pI 7.0) was detected only in spermatozoa. (Table 1)

Condensing spermatids were incubated with [35S]methionine to determine whether the new proteins were being synthesized at this stage. Hsc71, hsc74 and P70 were present on Coomassie blue-stained 2D gels of condensing spermatid proteins, but were not detected on 2D autoradiograms of [35S]methionine-labeled spermatid proteins. In contrast, the proteins first detected in condensing spermatids did incorporate [35S]methionine, indicating that they were synthesized during this stage of spermatogenesis. Thus, the regulation of expression of these
new proteins seem to differ from that of hsc71, hsc74 and P70. Hsp70-like transcripts unique to the testis have been detected in mice (17) and rats (9). One transcript first appears in haploid spermatogenic cells and is stable throughout spermiogenesis (17). This transcript may give rise to one or more of the newly synthesized proteins recognized by antibody 13D3 in condensing spermatids and present in spermatozoa.

III. INTRACELLULAR LOCATION OF PROTEINS RECOGNIZED BY 13D3

Proteins recognized by 13D3 were distributed throughout the cytoplasm of pachytene spermatocytes and round spermatids. This is consistent with previous observations of the cytoplasmic localization of hsc71 in unstressed cells (10,16). However, immunofluorescent labeling with 13D3 was restricted to the postacrosomal regions in condensing spermatids and to the midpiece in spermatozoa. The restricted distribution of immunofluorescence occurs coincidently with the appearance of the new proteins. Although the function of the new proteins is not known at the present time, their stage-specific appearance suggests that they serve a role in the terminal differentiation steps of male germ cells and/or in the function of the mature spermatozoon.

We have demonstrated recently that spermatogenic cells synthesize another hsp70-like protein (P70) during a specific stage of spermatogenesis (2). Together with the present findings, these data suggest that genes closely related to the HSP70 multigene family are regulated in a unique fashion during spermatogenesis, and that their products may have important functions during this process.
REFERENCES