# STAGE-DEPENDENCY OF PROTEINS AND GELATINOLYTIC PROTEINASE ACTIVITIES DURING SPERMATOGENESIS

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Spermatogenesis is an unique developmental process involving profound changes in number and shape of germ cells and associated somatic elements (Sertoli cells). Presumably, it requires the turning on and off of different genes in a strict temporal and cell-specific order, which should be reflected in changes in the protein products of gene expression. Due to the complex organization of the mammalian testis, it is not feasible technically to analyze gene expression or protein synthesis stage-by-stage in intact germinal units. In the testis of the spiny dogfish (Squalus acanthias), however, the primary germinal units (spermatocysts: isogenetic germ cell clones plus stage-synchronized Sertoli cells) are anatomically distinct and can be isolated intact (Callard et al., J. Exp. Zool. Suppl.2: 23, 1989). Moreover, spermatocysts are arranged in maturational order across the diameter of the testis, facilitating analysis of proteins in relation to specific developmental stages. We report here initial studies in which we document protein composition of premeiotic (PrM), meiotic (M) and postmeiotic (PoM) spermatocysts.

Testes of 3-5 dogfish were dissected according to stage of development and spermatocysts isolated according to procedures described by Dubois & Callard (J. Exp. Zool. 258: 359, 1991). To solubilize proteins, cysts (20 $\mu$l packed volume) were boiled for 30 min in 30 $\mu$l 20% SDS and an additional 10 min after adding 270 $\mu$l buffer (0.065 M Tris-HCl, 10% glycerol, 2% ß-mercaptoethanol, 0.04% bromophenol blue). After centrifugation for 15 min at 12,000 x g, protein concentrations were determined in TCA-precipitated aliquots by the method of Lowry et al. (J. Biol. Chem. 193: 265, 1951). Proteins (100 $\mu$g/well) were separated by SDS-PAGE (Laemmli, Nature 277: 680, 1970) using 4-20% linear gradient gels with a 4% stacking gel, followed by Coomassie blue staining. Apparent molecular weights (Mr) of stained bands were estimated by linear regression analysis using known molecular weight markers as standards. The relative abundance of a given protein band was estimated visually by staining intensity using a scale of 0 to +++, with + being assigned to the stage of lowest intensity and 0 indicating no detectable staining. In addition, comparisons were made of all proteins in a given stage, and those of greatest intensity indicated.

The stage-related distribution of 13 representative proteins of a total of 30-35 observed bands is summarized in Table 1 for spermatocysts isolated in July versus September, and results were similar for at least 4 other preparations. Most protein bands were present in all stages but each displayed a unique stage-dependent pattern. For example, in July the 28.6 kDa band was PrM > M > PoM, whereas the 109.8 kDa band was PoM > M = PrM and the 54.8 kDa band was M > PrM > PoM. Only a few bands were stage-specific (e.g. 90.8 kDa in PoM stages only) or were equivalent in all stages (e.g. 83.9 kDa band). Comparing spermatocysts from July versus September, which represent the progression toward spermatogenic inactivity, some changes were detectable (e.g. the 35.7 kDa band appears only in September), but the changes were minor for most protein bands.

Table 1. SDS-PAGE analysis of proteins in premeiotic (PrM), meiotic (M) and postmeiotic (PoM) spermatocysts
The grading (0,+,++,+++) reflects the intensity of a given protein across all three developmental stages. The most abundant proteins within each stage are marked with *.

| Apparent Mr kDa | July | | | September | | |
|---|---|---|---|---|---|---|
| | PrM | M | PoM | PrM | M | PoM |
| 109.8 | + | + | ++ | + | + | ++ |
| 98.3 | ++ | ++ | + | ++ | ++ | + |
| 90.8 | 0 | 0 | + | 0 | 0 | + |
| 72.8 | + | ++ | + | 0 | ++ | + |
| 83.9 | + | + | + | 0 | + | + |
| 54.8 | ++* | +++* | +* | ++* | ++* | +* |
| 44.6 | + | + | + | + | + | + |
| 35.7 | 0 | 0 | 0 | + | + | 0 |
| 28.6 | +++ | ++ | + | +++* | ++* | + |
| 25.2 | 0 | + | + | + | ++ | +++* |
| 16.5 | ++* | ++* | + | ++ | ++* | + |
| 15.7 | ++* | ++* | + | ++* | ++* | + |
| 14.5 | ++* | ++* | + | ++ | ++* | + |

Because the profound remodeling of both germ cells and Sertoli cells which occurs during spermatogenesis might involve limited proteolysis, we used the technique of gelatin-containing SDS-PAGE zymography (Heussen & Dowdle, Anal. Biochem. 102: 186, 1980) to determine whether any of the observed protein bands were associated with proteinase activity. In this procedure, proteinase activity is indicated on electrophoretograms as clear bands on a uniformly amido black-stained background of gelatin. We found proteinases with apparent Mr of 104 kDa, 81 kDa and 69 kDa in all three spermatogenic stages and each was uniquely stage-related. The 104 kDa proteinase showed the same intensity throughout all three stages, while the activity of the 81 kDa proteinase was highest in PrM and PoM cysts. The enzymatic activity of the 69 kDa proteinase was clearly visible in PoM cysts but very low in the other stages; however, this distribution of activity varied between different cyst preparations. It remains to be determined whether these proteinase activities correspond to Coomassie blue stained bands with Mr of 109.8 kDa, 83.9 kDa and 72.8 kDa.

Initial results demonstrate the feasibility of this approach for analysis of changes in cyst protein composition during spermatogenesis and as a first step in identifying specific proteins and their genes. Furthermore, because staged spermatocysts are amenable to culture for up to 7 days (DuBois and Callard, J. Exp. Zool. 258: 359,1991), future experiments will allow radiolabeling of synthesized proteins directly and the testing of putative regulators of gene expression (e.g. steroids and other hormones, growth factors) under defined conditions in vitro.

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