STAGE-RELATED AND REGULATED CHANGES IN PROTEIN SYNTHESIS DURING SPERMATOGENESIS IN VITRO: THE SHARK (SQUALUS ACANTHIAS) TESTIS MODEL

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In shark testis, the basic germinal unit is a closed, spherical spermatocyst comprising a single germ cell clone and a second clonal population of stage-synchronized Sertoli cells. Thus it is amenable to isolation and culture without disrupting cellular interrelationships and the specialized germinal compartment. Also, spermatocysts are arranged in maturational order across the diameter of the testis, which facilitates isolation of defined stages. As part of an ongoing program of research, we are using shark spermatocysts to document stage-related growth and differentiated traits during spermatogenesis in vitro and to identify intrinsic and extrinsic mechanisms controlling the developmental progression. We previously reported that cyst protein composition is quantitatively and qualitatively unique in different stages (Betka et al., Bull. MDIBL 31:47, 1992). Here we describe initial studies using protein synthesis to assess temporal and regulator-induced changes in gene expression. Spermatocysts in premeiotic (PrM), meiotic (M) and postmeiotic (PoM) stages were isolated and cultured as previously described (DuBois & Callard, J. Exp. Zool. 258:359, 1991), and regulators known to affect DNA synthesis, steroidogenesis, or programmed cell death in the same system were screened for their effects on protein synthesis. The following were added at seeding: insulin/transferrin/selenite (ITS, 10 μg-10μg-10 ng/ml); 3-isobutyl-1-methylxanthine (IBMX, 1 mM); dibutyryl cAMP (dbcAMP, 10 mM); calcium ionophore (A23187, 0.5 μg/ml); tumor promoting agent (TPA; phorbol-12,13-dibutyrate, 1 μM); all trans-retinoic acid (1 μM). After 2 d, [^{35}S]methionine (5 μci/ml) was added for an additional 24 h, [^{35}S]labeled amino acid uptake vs. incorporation into acid-insoluble macromolecules was quantified, and protein banding patterns on SDS-PAGE gels after fluorography were analyzed by visual inspection. All lanes had 25,000 cpm.

Methionine uptake was similar in PrM and PoM cysts (respectively, 972 ± 202 and 1241 ± 123 cpm/μg protein ± SD, n = 4) but significantly higher than in M cysts (552 ± 32 cpm/μg). The same stage-related pattern was observed when labeled amino acid incorporation into protein was measured: PrM and PoM cysts were similar (391 ± 98 and 379 ± 34 cpm/μg) but higher than M stage cysts (152 ± 25 cpm/μg). Figure 1 shows that effects of a given regulator on total amino acid uptake and incorporation differed by stage. For example, retinoic acid markedly stimulated both parameters in PrM and M stages but not in PoM stages. IBMX and dbcAMP, alone or in combination, decreased protein synthesis in all stages but effects were significant only in M and PoM cysts. PMA and A23187 decreased protein synthesis in PoM stages only. Figure 2 shows that synthesis of specific proteins by spermatocysts in basal medium is distinctly stage-dependent. For example, labeling intensity of 42 kDa (p42) and 102 kDa (p102) bands increased progressively through maturation (PoM > M >> PrM), whereas an 80 kDa (p80) band was seen in PoM cysts only. When synthesized proteins from different treatment groups were analyzed by SDS-PAGE, no changes in banding patterns were observed with ITS, TPA or retinoic acid despite changes in overall labeling rates; however, IBMX and dbcAMP increased labeling intensity of two bands in PrM cysts (p42, p68) and decreased p72 in PrM cysts and p102 in PrM and PoM cysts (Table 1). A23187 affected the same bands, but the direction of change and/or responsive stage was unique to this regulator.

Some of the [^{35}S]methionine labeled bands seen in the present study corresponded to Coomassie blue-stained bands in our previous study (e.g., 72 kDa), and to electrophoretic mobilities of known proteins (e.g. actin); however, it would be premature to draw conclusions as to protein identity. Nonetheless, results show that shark spermatocysts have utility for differential analysis of proteins by stage of development and in response to regulators under defined conditions in vitro, and in this respect, are superior to currently available mammalian systems. In future studies, this approach can be used to screen putative regulators and to optimize induction conditions for direct analysis of differentially expressed mRNAs by plus/minus screening, differential hybridization and PCR methodology. (Supported by NIH HD16715)

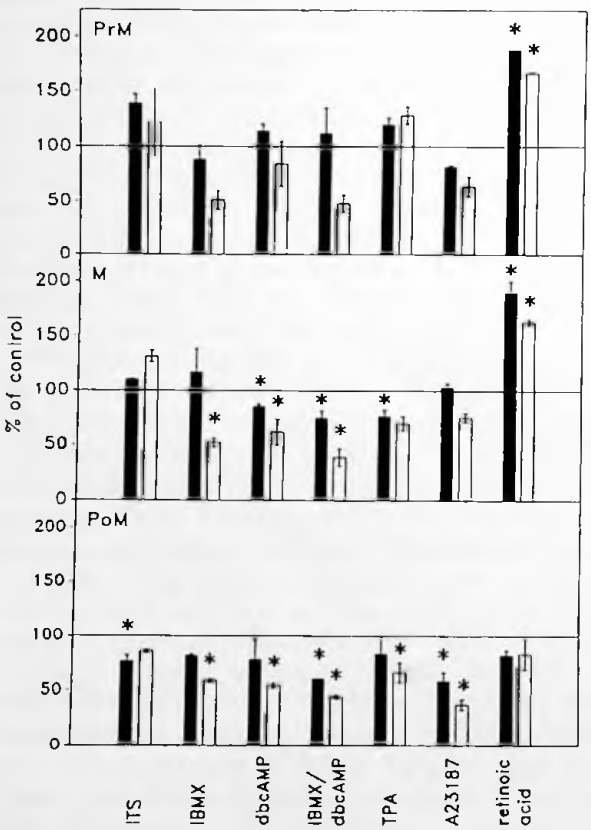

Figure 1: Effects of regulators on uptake (■) and incorporation (□) of [^{35}S]methionine by premeiotic (PrM), meiotic (M) and postmeiotic (PoM) spermatocysts. Data are expressed as % control ± SD. The actual values of treatments (n = 2) were tested against controls (n = 4) within each stage by Student's t-test and significant differences (P, <0.05) indicated by asterisks.

Table 1: Summary of regulator effects on specific protein synthesis by cultured cysts.

Protein (kDa)	Regulation IBMX/dbcAMP	A23187
p42	PrM ↑	PoM ↑
p68	PrM ↑	PoM ↓
p72	PrM ↓	PrM ↓
p80	→	PrM ↑ PoM ↓
p102	PrM ↓ PoM ↓	PrM ↑ PoM ↑

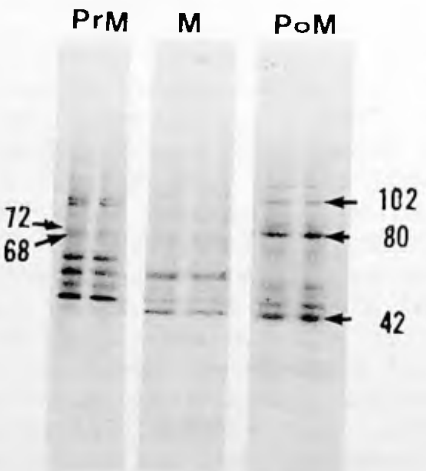

Figure 2: SDS-PAGE analysis of [^{35}S]labeled proteins synthesized by staged spermatocysts in basal medium. Arrows indicate bands affected by regulators (see Table 1).