PARACRINE REGULATION OF PREMEIOTIC GERM CELL PROLIFERATION IN THE TESTIS OF THE SPINY DOGFISH, SQUALUS ACANTHIAS

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The regulation of spermatogenesis is a complex process involving functional interactions among several cell types. Studies trying to elucidate the underlying mechanisms are made difficult by the complex organization of the mammalian testis. In contrast, the dogfish shark, Squalus acanthias, has testes in which germ cells plus associated somatic cells form discrete anatomical units (spermatocysts) and are arranged in maturational stages across the diameter of the testis. Using the shark testis model (see Callard et al., 1989, J. Exp. Zool., Suppl. 2: 353-364, for review), it was shown that a basic cell function, DNA synthesis, can be maintained quantitatively for several days under in vitro conditions and is stage-dependent. DNA synthesis by premeiotic (PrM) spermatocysts is 6-fold higher than that observed in meiotic (M) and postmeiotic (PoM) spermatocysts (DuBois and Callard, 1991, J. Exp. Zool., 258: 359-372). As part of studies in which we are surveying possible regulators of DNA synthesis in PrM cysts, we tested the hypothesis that more mature developmental stages, which are upstream in the vascular pathway within the testis, may signal the developmental advance of less mature stages.

PrM spermatocysts were cultured in the inner well of a two-chamber microwell assembly for 48h (t_0 to t_{48}) in the presence of medium alone or with PrM, M or PoM spermatocysts in the outer well. DNA synthesis was measured from t_{24} to t_{48} by the incorporation of [methyl-3H] thymidine into trichloroacetic acid (TCA) precipitable material. Radioactivity per unit cyst protein (micro-Lowry) was not significantly altered when PrM spermatocysts were cocultured with either PrM or M spermatocysts but decreased by one-half in the presence of PoM spermatocysts (Table 1A). The inhibition of DNA synthesis in PrM cysts caused by PoM cysts was confirmed in a dose-response study. Coculture of PrM cysts with 1 mg excess cysts of the same stage (PrM) reduced DNA synthesis at most to 66% of the control values. In contrast, an excess of 0.5 mg PoM spermatocysts reduced DNA synthesis in PrM spermatocysts to 12% of controls (Table 1B).

Further investigations tested the specificity of the response by including other tissues. DNA synthesis was not affected when PrM cysts were cocultured with different amounts of muscle, epididymis or PrM fragments, whereas fragments of PoM tissue and epigonal organ (a lymphomyeloid tissue encapsulating the testis immediately adjacent to the PoM region) markedly reduced DNA synthesis in PrM cysts. These results were confirmed in a second, similar experiment and also when tissue extracts rather than tissue fragments were added to PrM cysts. Further, in contrast to the inhibitory effects of testicular or epigonal tissue, a powerful stimulatory effect on PrM DNA synthesis was observed when extracts of spermatozoa collected from the sperm sac were tested.

Table 1. Regulation of DNA synthesis in premeiotic (PrM) spermatocysts.

A) Effects of coculture with premeiotic (PrM), meiotic (M) and postmeiotic (PoM) spermatocysts (dpm/ug protein ± SEM of two separate experiments, each in duplicate)

Medium (Ctrl)	+PrM cysts	+M cysts	+PoM cysts
1260±75	1080±115	965±160	615±57

B) Dose-response effects of PrM, M or PoM spermatocysts (percentage of control in two separate experiments each in triplicate)

	Excess protein (ug)	% DNA synthesis
PrM cysts	0.0	100.0
	115.0	75.5
	282.0	67.3
	555.5	68.2
M cysts	0.0	100.0
	263.5	47.5
	470.0	32.7
	1055.5	33.6
PoM cysts	0.0	100.0
	261.0	23.3
	466.5	12.0
	2247.5	19.2

Our findings, therefore, point to the existence of one or more substances present in the PoM region of the testis and in the epigonal organ which are capable of inhibiting the synthesis of DNA in PrM stages of spermatogenesis. These results are consistent with an early report of an inhibitory chalone in rat testis (Clermont and Mauger, 1974, Cell Tissue Kinet., 7: 165-172); however, the latter has not been further studied. Also our test system has identified a stimulatory substance associated with spermatozoa after spermiation. These stimulatory and inhibitory effects support the existence of a paracrine mechanism for the regulation of mitotic proliferation of germ cells during the early stages of spermatogenesis and provide a useful assay for isolation and purification studies of putative regulators.

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