# REGULATION OF DNA AND PROTEIN SYNTHESIS IN THE DOGFISH (SQUALUS ACANTHIAS) TESTIS

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Spermatogenesis depends on the sequential development of germ cells from undifferentiated stem cells to mature spermatozoa. In the dogfish (Squalus acanthias) testis, this developmental sequence is organized into a morphologically discernible spermatogenic wave, in which germ cells proliferate, develop and migrate through the testis in a precise manner. Moreover, the germ cells and their associated somatic Sertoli cells are organized into discrete spermatocysts each of which is composed exclusively of synchronous clones of the two cell types. Thus, the morphology of the Squalus testis offers some distinct advantages for the study of vertebrate spermatogenesis that are not available in the classical mammalian model systems. First, various developmental stages of germ and Sertoli cells can be easily identified and isolated simply by their position relative to certain anatomical landmarks and their appearance under the dissecting microscope (Callard et al., Endocrinology 117:1328, 1985). In our laboratory we commonly divide the testis into three zones, readily visible to the naked eye in situ, to compare stage-related biochemical characteristics. These zones, labelled I, II, and III, correspond to premeiotic, meiotic, and postmeiotic stages, respectively. Second, the cystic mode of proliferation allows for the isolation and culture of intact, functional spermatogenic units. Morphological and biochemical descriptions of the Squalus testis model have been summarized by Callard et al. (J. Exp. Zool. Suppl. 2:23, 1989). In this report we describe the effects of some potential regulators on the synthesis of DNA and protein by cultured spermatocysts isolated from Squalus testes.

For each experiment, spermatocysts were isolated from testes of 2-4 sharks and maintained in culture at 17 (DNA) or 20°C (protein) as described by Callard and Dubois (MDIBL Bull. 27:30, 1988). For analysis of DNA synthesis we cultured cysts in Leibovitz L-15 medium; for protein synthesis we used methionine-deficient Minimum Essential Medium; both media were modified for use with elasmobranch tissue as noted in Callard and Dubois (op. cit.) except that bovine serum was not added. After various periods of treatment with regulators, cysts were incubated with 5 uCi/ml $^{3}$H-thymidine (DNA) or $^{35}$S-methionine (protein) for 24 (DNA) or 48 hr (protein) before harvesting the cysts. Harvested cysts were washed and centrifuged at low speed twice with saline solution augmented with excess unlabelled thymidine or methionine. Then, cysts were treated with ice-cold 12% trichloroacetic acid for 10-15 min followed by centrifugation at 3000 g for 15-30 min at 0-4 °C. The pellet was washed again and then solubilized in 0.2 M NaOH. Aliquots of the solubilized pellet were analyzed for protein content by the method of Lowry and for radioactivity. Results from cysts treated with regulators were compared to those from untreated control groups by an unpaired t-test with a pooled variance estimate.

Initial experiments showed that most DNA synthesis, as indicated by the uptake of $^{3}$H-thymidine, occured in zone I, whereas synthesis in zones II and III was about 10% and 1%, respectively, of that in zone I (Fig. 1a). Treatment with bovine insulin (10 ug/ml) almost doubled DNA synthesis in zones I and II, but not in zone III. Treatment with a combination of dibutyryl cyclic AMP (1 mM, db-cAMP) and 3-isobutyl-1-

methyl-xanthine (1 mM, IBMX) decreased DNA synthesis by more than half in all zones. Mammalian gonadotropin releasing hormone (mGnRH,100 ng/ml) slightly (22%) but significantly (p=0.04) increased DNA synthesis in zone III. Retinoic acid (0.5 and 1.0 uM) had no effect on DNA synthesis. Protein synthesis as indicated by the incorporation of $^{35}$S-methionine also varied between zones (I>III>II; Fig. 1b). Treatment with the regulators listed above caused slight, and usually insignificant, effects on protein synthesis in all zones.

In subsequent experiments we focused only on the effects of regulators on DNA synthesis in zone I. We were particularly interested in the possible role of estradiol since it is known that zone I cysts have the highest concentration of estradiol receptors relative to other zones, but a definitive role for estradiol in this zone is not yet known (Callard et al., op. cit.). We conducted three separate experiments to assess a dose-responsive effect of estradiol on DNA synthesis. In one experiment we exposed cultured cysts to graded concentrations of estradiol between 0.1 and 1000 nM for 48 hr beginning at the time of seeding; labelled thymidine was added after 24 hr. In another experiment we "starved" cysts in culture for 5 days and then added graded doses of estradiol for 48 h and labelled thymidine for 24 hr before harvesting. To test possible cumulative effects, we cultured cysts in the presence of 100 nM estradiol for 1, 2, 3, 5, and 7 days before harvesting after a 24 h exposure to labelled thymidine. Other cysts were treated with bovine insulin (10 ug/ml) as a positive control to assess the cysts ability to respond to a known promoter of DNA synthesis. Cysts treated with insulin showed the expected doubling of DNA synthesis; however, in no case did estradiol treatment alter synthesis relative to untreated control cultures. In another experiment, 100 nM estradiol failed to affect insulin-stimulated DNA synthesis. A possible regulatory role for estradiol with respect to DNA synthesis in zone I cysts is not supported by our experiments.

We next turned our attention to the marked inhibitory effects of db-cAMP and IBMX in combination on DNA synthesis. Employing a short-term 48 h experiment, we treated zone I cysts with db-cAMP in graded doses (0.01, 0.1, 1.0 mM) with and without 1.0 mM IBMX during the entire culture period. Alone db-cAMP had a slight stimulatory effect at the two highest doses; however, the addition of IBMX decreased DNA synthesis by more than half in all cases. We conducted the reciprocal experiment, varying the concentration of IBMX (0.01, 0.1, 1.0 mM) with and without db-cAMP (1.0 mM); but, in this experiment we did not begin treatment until the fifth day in culture, harvesting on the seventh. Alone, IBMX decreased DNA synthesis as dosage increased from 0.01 to 1.0 mM (83, 70, 21% of the control value, respectively). The addition of db-cAMP amplified the inhibition for the two lower doses (59, 33, 23% of the control value, respectively). The effect of IBMX is not mimicked by related methylated analogs, including those of adenine, adenosine, xanthine, and hypo-xanthine. Adenosine triphosphate (1 mM) did inhibit DNA synthesis, but less profoundly than did IBMX.

Our experiments lend additional support for the use of Squalus testis as a model system for the study of vertebrate spermatogenesis. It is evident from our results that cell proliferation, as indicated by the uptake of exogenous thymidine, is most active in zone I spermatocysts, supporting previous morphological descriptions of the spermatogenic process in Squalus. Further, our results demonstrate that certain substances can dramatically increase or decrease the rate of DNA synthesis in cultured spermatocysts. These findings will serve as the basis for future studies on the regulation of cell proliferation in Squalus testis.

[Supported by a Pew Undergraduate Faculty Fellowship to J.M.R. We thank Dr. Wilfrid Dubois for valuable advice.]

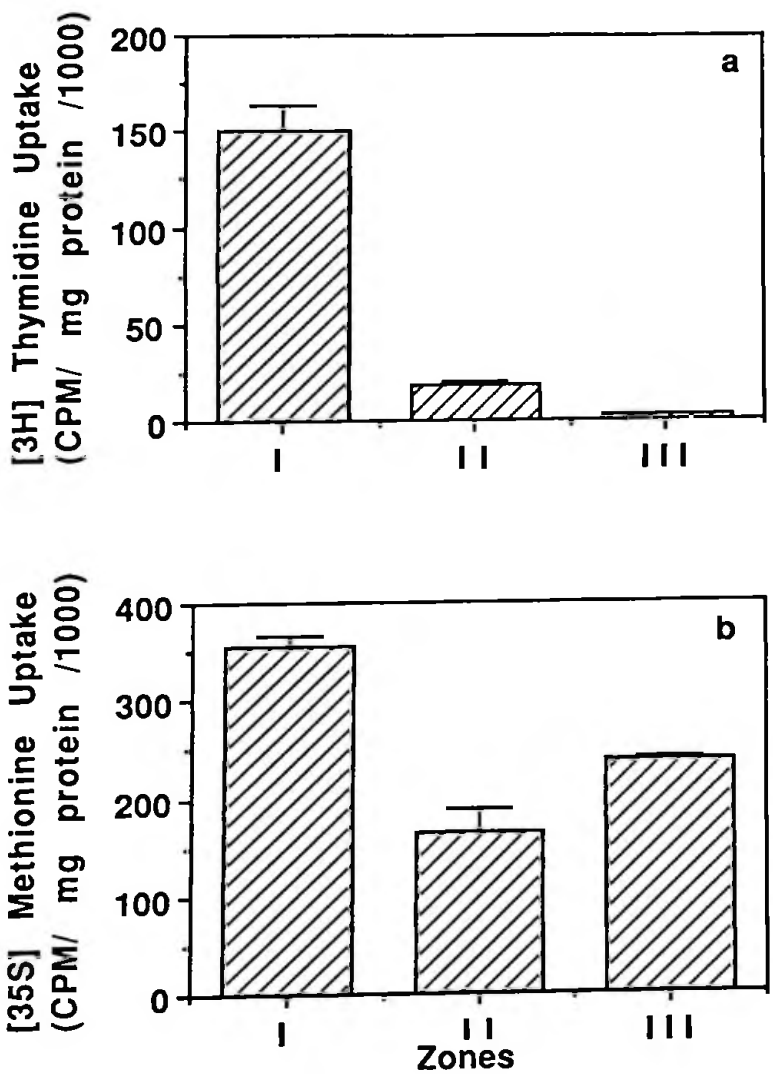


Fig. 1. $^{3}$H-thymidine (panel a) and $^{35}$S-methionine uptake (panel b) by zone in Squalus testis. Spermatocysts were cultured for 7 (a) or 8 (b) days before harvesting. Values represent the mean + SEM of 3 (b) or 4 (a) cultures.