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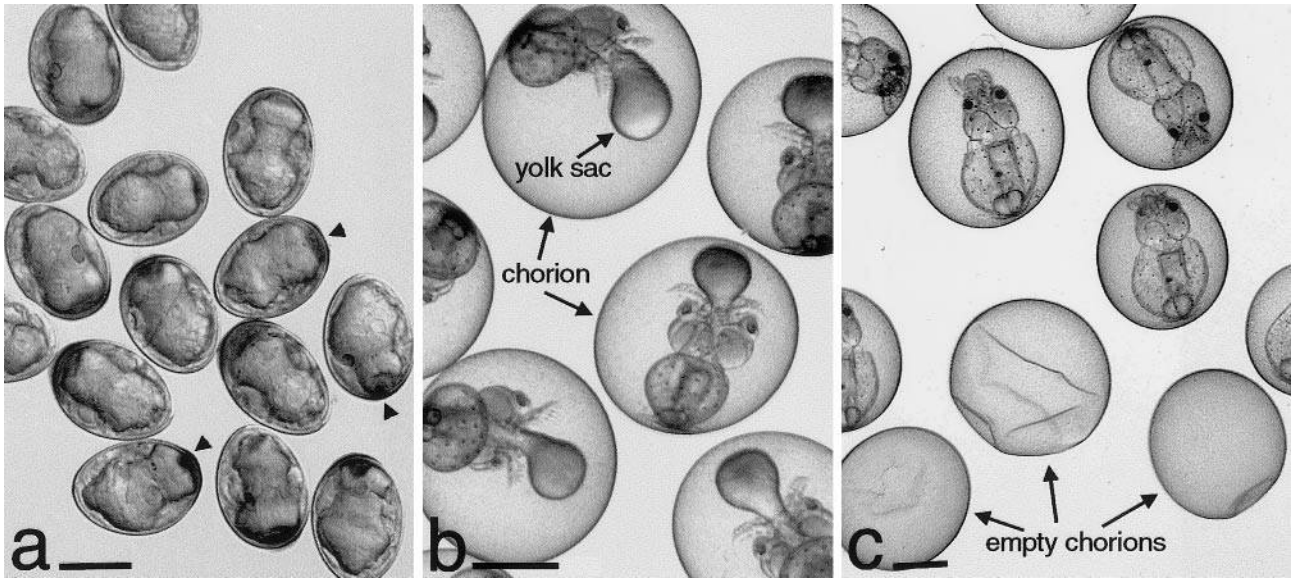

Figure 1. *Bovine serum albumin enhances chorion expansion to permit normal development and hatching of* in vitro *fertilized squid embryos. (a) Eighteen-day-old embryos cultured in MFSW alone. Note: the chorion has not expanded to permit normal development. Arrowheads indicate deteriorating yolk sac tissue. (b) Eighteen-day embryos cultured in 0.5% BSA/MFSW permits chorion expansion and normal development. (c) Twenty-one-day embryos, hatchling stage, cultured in 0.5% BSA/MFSW. Arrows indicate three empty chorions recently discarded by hatchlings. Scale bars = 1000 μm.*

embryo culture enables us to treat cephalopod embryos with chemical agents, such as lithium chloride (11) or retinoids (12), that have been shown to predictably alter development in other organisms, thereby enhancing our ability to work with and understand the embryos of this evolutionarily old and successful group of organisms.

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mRNAs Located in *Squalus acanthias* (Spiny Dogfish) Oligodendrocyte Processes

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This study identifies mRNAs located in oligodendrocyte (OL) processes of spiny dogfish and compares them with mRNAs that were previously identified in rat OL processes (1, 2). Because these two species have independent evolutionary histories covering hundreds of millions of years, mRNAs common to OL processes in both species likely represent proteins recruited to form the original myelin sheaths in the common gnathostome ancestor. Of the mRNAs present in rat OL processes, myelin basic protein (MBP) and myelin-associated oligodendrocytic basic protein (MOBP) mRNAs dominate (1, 2). Surprisingly, however, MBP

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mRNA is not located in spiny dogfish OL processes; rather it is restricted to OL soma as measured in both *in situ* hybridization and subcellular fractionation experiments (Gould, unpubl.). Thus, MBP synthesis must have moved from the OL soma to its processes sometime after the mammalian line diverged from elasmobranches.

To identify mRNAs in spiny dogfish OL processes, we applied the method we developed to identify mRNAs in rat OL processes. This method takes advantage of the finding that mRNAs located in OL processes are selectively trapped in myelin vesicles during homogenization of brain tissue (1–3). As starting material for subtractive hybridization, RNA was obtained from low-speed supernatant, myelin, and pellet fractions prepared from adult (4) and fetal (20–22 cm) spiny dogfish brains. Fetal spiny dogfish brains were used because they have a greater ratio of OL process cytoplasm to myelin sheaths than adult brains. In initial studies, we obtained results suggesting that adult spiny dogfish myelin was contaminated with non-OL process mRNAs (see below). Brains were removed, homogenized in 0.85 *M* (adult) or 1 *M* (fetal) sucrose with 10 m*M* HEPES, pH 7.4, 3 m*M* dithiothreitol (DTT) (5). The homogenate was centrifuged at 4000 rpm for 10 min at 4 °C to pellet nuclei and cell debris. LSS was transferred to an ultracentrifuge tube, overlaid with 0.25 *M* sucrose in HEPES/DTT, and centrifuged at 26,000 rpm for 3.5 h at 4 °C in a SW28 rotor. Myelin (white material accumulated at the 0.25 *M* sucrose/0.85 or 1 *M* sucrose interface) and pellet were transferred to separate tubes, diluted with 10 m*M* $MgCl_2$, and centrifuged at 12,000 rpm for 10 min at 4 °C. Total RNA was extracted from LSS, myelin, and pellet samples with Tri Reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's instructions. For studies with adult tissue, low-speed supernatant (LSS) was used to prepare driver for subtractive hybridization. For studies with fetal tissue, the 1 *M* sucrose pellet was used to prepare driver. Poly A RNA was prepared (MicroPolyA Pure™ kit, Ambion, Austin, TX) and used for suppression subtractive hybridization (PCR-Select™ Subtractive Hybridization kit, BD Clontech, Palo Alto, CA) according to the manufacturer's instructions. PCR products were subcloned into pGEM T-easy vector (Promega, Madison, WI) and sequenced on Li-Cor and ABI 3700 sequencing machines in the Josephine Bay Paul Center. Sequences that matched GenBank non-redundant (NR) nucleotide entries were identified using the Basic Local Alignment Search Tool (BLASTN) (http://www.ncbi.nlm.nih.gov) (6).

For the adult studies, over 150 sequences were analyzed (4). Twenty-five that had matches in the GenBank non-redundant database were analyzed by Northern blot for enrichment of mRNA in myelin *versus* LSS. None of the cDNAs identified mRNAs that were enriched in myelin, probably because the abundant myelin in adult brain caused non-specific trapping of mRNAs present at other neural locations. With the fetal brain subtraction, 280 sequences were analyzed. Over half (148, 53%) were derived from the *Squalus acanthias* mitochondrial genome. Another 73 (26%) did not match any sequence in the GenBank non-redundant database. We will determine if any of these sequences match entries in GenBank using BLASTN (EST database) and BLASTX (NR database) as well as the pufferfish (http://fugu.hgmp.mrc.ac.uk/) and the zebrafish (http://www.ensembl.org/Danio_rerio/blastview) databases. The remaining 59 (21%) sequences matched entries in the GenBank NR database. Fifteen different ribosomal protein mRNAs were identified as well as three elongation factors (1α, 2 and 5α); all are important for translational control of OL process protein synthesis. We previously identified four different ribosomal protein mRNAs and two different elongation factor mRNAs in rat OL processes (2, 7). Together, these results suggest that local regulation of OL process protein synthesis was an original property. Some of these were larger than known ribosomal protein and elongation factor mRNAs (Gould, unpubl.), indicating that they either code for different proteins or contain untranslated regions that target them to OL processes. In an independent study, the mRNA for ribosomal protein L4 was identified in PC12 neurites (8), a finding consistent with local translational control being a property of process-based protein synthesis. As in rat myelin, spiny dogfish myelin contains ferritin heavy chain mRNA. This result suggests that iron storage in OL processes was locally regulated early in evolutionary time. None of the other 18 known mRNAs identified here matched mRNAs identified in rat OL processes. Among the mRNAs of particular interest are homologues of sec61 (accession number BC019158), proteosome protein (BC003197), calmodulin-2 (NM_001743) and epsilon-coatamer (AB042117), all proteins with potential function in membrane biogenesis. We will use both Northern blot and subtractive hybridization efficiency analysis to confirm that these cDNAs represent mRNAs located in spiny dogfish OL processes. As neither MBP or MOBP mRNAs were identified in these studies, our results indicate that these proteins were targeted to OL processes after mammalian ancestors diverged from elasmobranches.

Because numerous mitochondrial genes and limited numbers of other genes were identified in this study, we are conducting additional studies with myelin and pellet fractions prepared by subjecting fetal spiny dogfish brains to hyperosmotic conditions (1.4 *M* sucrose) during homogenization and hypotonic conditions (0.85 *M* sucrose) just prior to density gradient centrifugation. We already know that when rat brain is homogenized in hyperosmotic sucrose the variety of cDNAs obtained by subtractive hybridization is increased in comparison with homogenization in iso-osmotic sucrose (2, 7). In ongoing studies, we lowered the sucrose interface to 0.85 *M* sucrose. Future studies are planned to determine which of the mRNAs identified in rat OL process mRNAs are expressed in spiny dogfish OL processes and *vice versa.*

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