tensity than most techniques, which can be of critical advantage for viewing light-sensitive specimens. This method of image formation does not require any system of rings, slits, or half stops. Therefore, it also avoids halos or diffraction fringes arising from the presence of abrupt edges in the path of illumination (*6*) as in phase-contrast microscopy (*4*). Video-enhanced AIC is also especially suitable for observation of mixed phase and amplitude objects such as cytochemically labeled specimens (Fig. 3, g and h). Weakly labeled structures can easily be seen (Fig. 3h), and clear imaging of any other focal plane in the specimen (Fig. 3g) can be obtained with simple readjustments of the camera's gain and black level.

The video-enhanced AIC technique results from the application of video-derived contrast enhancement to an optimized procedure of imaging transparent objects with oblique rays of illumination. With this combination, images with the highest resolution and exceptionally broad gray scale can be obtained. This technique has several advantages over other video light microscopy techniques and is simple, depending only on minor adjustments to currently available standard equipment. These advantages increase the versatility and range of application of video-enhanced light microscopy to virtually any type of specimen.

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Effects of Extracellular Egg Factors on Sperm Guanylate Cyclase

Abstract. *Extracellular factors from the sea urchin egg induce a change in the electrophoretic mobility of an abundant sperm membrane phosphoprotein. The modified protein was identified as guanylate cyclase. The mobility shift of the cyclase was shown to be associated with a decrease in its enzymatic activity.*

Most animal eggs are surrounded by extracellular investments through which sperm pass before contacting the egg surface. These extracellular coats contain molecules that profoundly affect sperm physiology (*1*). The sea urchin egg has a glycoprotein jelly coat that triggers the acrosome reaction (*2*), increased respiration (*3, 4*), and increased motility (*5, 6*) of sperm. The complete sequence of molecular events underlying sperm activation by egg jelly has not been elucidated, although changes in ion flux (*7–10*), membrane potential (*11*), cyclic nucleotide metabolism (*1, 12*), and a number of enzymatic activities (*13–18*) have been described.

We reported earlier that egg jelly induces a change in the electrophoretic mobility of a major membrane protein in *Arbacia punctulata* sperm, from an apparent molecular mass of 160 kilodaltons (kD) to 150 kD (*19*). We now report that the protein is guanylate cyclase, and we show that there is a correlation between the electrophoretic mobility of the protein and its enzymatic activity.

Sea urchin sperm have the highest activity of guanylate cyclase known for any cell (*1*). Isolation of particulate gua-

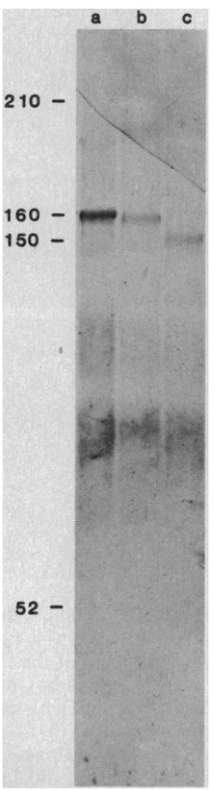

Fig. 1. Immunoblots showing cross-reaction between polyclonal antiserum to purified *S. purpuratus* sperm guanylate cyclase (*20*) and *A. punctulata* (a) sperm membranes (10 μg), (b) whole sperm (50 μg), and (c) whole jelly-treated sperm (50 μg). The numbers on the left denote apparent molecular mass in kilodaltons. Membranes were prepared as follows: dry *A. punctulata* sperm were diluted (2°C) into 20 volumes of cavitation buffer (480 m*M* NaCl, 10 m*M* $MgCl_2$, 10 m*M* KCl, 5 m*M* adenosine triphosphate, 20 m*M* benzamidine-HCl, 0.5 m*M* phenylmethylsulfonyl fluoride, 10 m*M* MES (*p*H 6.0). The sperm were pelleted (12 minutes at 4000*g*), suspended in 10 volumes of the same buffer, and subjected to nitrogen cavitation (*25*) at 400 pounds per square inch for 10 minutes in a Parr bomb (2°C). The cavitate was centrifuged (2°C) for 10 minutes at 8000*g*, then for 30 minutes at 11,000*g* (discarding the pellet each time). Membranes were pelleted from the second supernatant by centrifugation for 45 minutes at 75,000*g* (2°C). Sperm were treated with egg jelly as described (*19*). Trichloroacetic acid–insoluble protein was prepared and subjected to sodium dodecyl sulfate (Sigma L-5750)–polyacrylamide gel electrophoresis (SDS-PAGE) as described (*19*). Protein was transferred from the gel to nitrocellulose paper in 150 m*M* glycine, 20 m*M* tris (*p*H 8.3), 20 percent (by volume) methanol at 6 V/cm for 14 hours. The nitrocellulose strips were incubated with primary antibody at a dilution of 10^{-3}, and specific antibody binding was visualized by use of a second antibody conjugated with horseradish peroxidase (*26*).

nylate cyclase from sperm of *Strongylocentrotus purpuratus* has been reported (*20*). A polyclonal antiserum to this protein was shown to be monospecific on immunoblots of detergent-extracted *S. purpuratus* sperm membranes (*20*). When this antiserum is used in immunoblots of *A. punctulata* sperm membranes, a single cross-reacting band is observed at 160 kD (Fig. 1a). When the sperm are treated with jelly, the cross-reacting band shifts from 160 to 150 kD (Fig. 1, b and c). This antiserum also inhibits more than 97 percent of the guanylate cyclase activity in homogenates of *A. punctulata* sperm (not shown). These results suggest that the 160-kD protein in *A. punctulata* sperm is guanylate cyclase.

The 150-kD form of this protein was isolated to near homogeneity (it has not yet been possible to maintain the protein in its 160-kD form during isolation). Briefly, sperm membranes were isolated by nitrogen cavitation and ultracentrifugation, solubilized in 0.5 percent Triton X-100, and applied to a concanavalin A–agarose affinity column. After overnight absorption and extensive washing of the material in the column the 150-kD protein was eluted with 300 m*M* α-methyl-D-mannoside in 1*M* NaCl (Fig. 2). Samples at each step of the purification were assayed for guanylate cyclase activity (Fig. 2). Specific activity was increased by a factor of 1760 in the final 150-kD fraction, confirming that the 150-kD protein is guanylate cyclase.

Homogenates of jelly-treated sperm containing various ratios of the 160- and 150-kD forms of the protein were prepared as described (*19*) and assayed for guanylate cyclase activity. The activity of homogenates containing the enzyme in the 160-kD form was 38 times higher (average for five experiments) than homogenates containing the enzyme in the 150-kD form (Fig. 3). Homogenates containing both forms of the enzyme showed intermediate activities (Fig. 3). The decrease in activity is not a result of inhibition by residual egg jelly; adding jelly to the assayed material had no effect on activity (Fig. 3F). A large decrease in activity in response to jelly was also observed when Mn^{2+} was replaced by Mg^{2+} in the assay buffer (not shown), although the absolute levels of Mg^{2+}-supported activity are considerably lower than those of Mn^{2+}-supported activity (*1*). Furthermore, treatment of sperm with 20 μ*M* monensin, which alone induces the 160- to 150-kD mobility shift of guanylate cyclase, also resulted in reduced guanylate cyclase activity in whole cell homogenates (not shown).

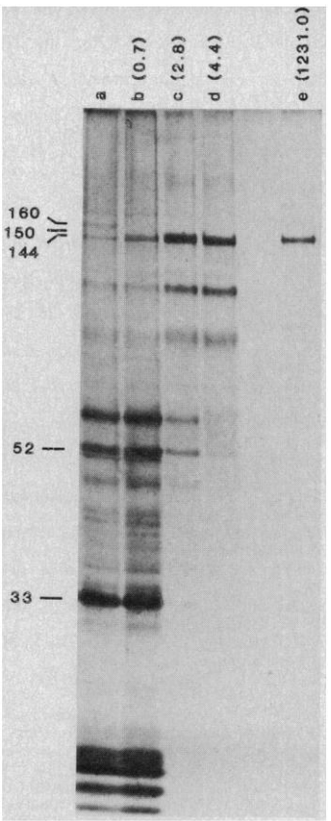

Fig. 2. Purification of the 150-kD protein: (a) untreated control sperm, (b) jelly-treated sperm, (c) membranes, (d) solubilized membranes, and (e) eluate from concanavalin A affinity column. Numbers in parentheses denote guanylate cyclase specific activity in nanomoles of cyclic GMP formed per minute per milligram. Numbers on left are apparent molecular mass in kilodaltons. Dry sperm (6 ml) were suspended in 40 ml of egg jelly (50 μg fucose equivalents per milliliter) (*27*) and incubated for 5 minutes at 21°C. The sperm were pelleted (12 minutes at 4000*g*), and membranes were prepared as described (Fig. 1). The membrane pellet was suspended in 10 ml of buffer A (0.5 percent Triton X-100, 20 m*M* MES at *p*H 6.2, 250 m*M* NaCl, 10 m*M* $CaCl_2$, 1 m*M* NaF, 1 m*M* dithiothreitol, 10 m*M* benzamidine-HCl, and 2.5 m*M* $MnCl_2$) and stirred gently for 20 minutes on ice. The solubilized membranes were centrifuged (2°C) for 45 minutes at 70,000*g*, and the pellet was discarded. The supernatant was added to 1.5 ml of concanavalin A–agarose affinity resin (*28*) and incubated for 12 hours on ice (with gentle stirring). The resin was washed with 300 ml of buffer A at 2°C and 20 ml of buffer A at 21°C. Bound protein was eluted at 21°C with buffer A, 300 m*M* α-methyl-D-mannoside, 750 m*M* NaCl. At each step in the procedure three portions were removed: one was made 50 percent (by volume) in glycerol and frozen at −70°C (*20*) for subsequent measurement of guanylate cyclase activity (*29*); one was used for fluorimetric protein determination (*30*); and one was precipitated in 10 percent (weight to volume) trichloroacetic acid and processed for SDS-PAGE (*19*). Protein (6 μg) was loaded in lanes a and b, 2.2 μg in lanes c and d, and 0.18 μg in lane e, and the gel was stained with silver (*31*). Prolonged electrophoresis, which effectively resolves the 150- and 144-kD proteins (*19*), confirmed that the eluted 150-kD fraction (lane e) contained no detectable 144-kD contamination.

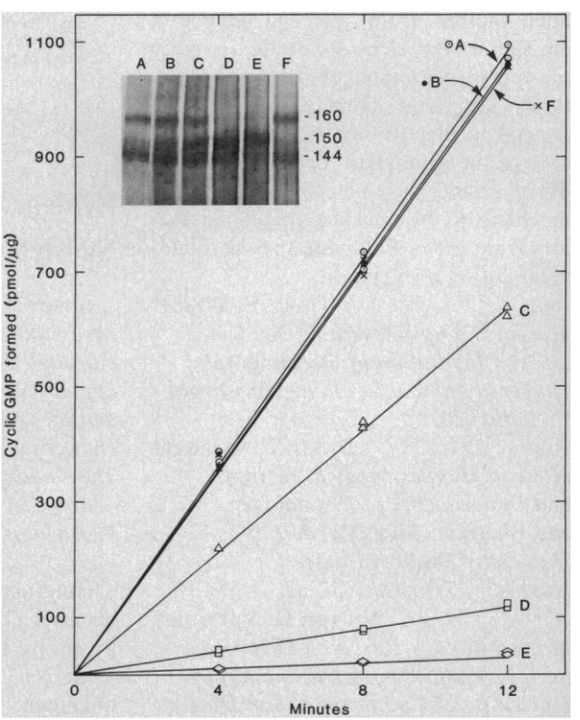

Fig. 3. *Arbacia punctulata* sperm were exposed to various concentrations of egg jelly assayed by fucose content (*27*): (A) no jelly (control); (B) 112 ng/ml; (C) 224 ng/ml; (D) 560 ng/ml; and (E) 1400 ng/ml. The sperm were then homogenized and assayed for guanylate cyclase activity. Curve F is a control in which egg jelly (fucose, 1650 ng/ml) was added to extract A. At the end of the 12-minute assay, portions were precipitated with trichloroacetic acid and analyzed by SDS-PAGE/silver stain (*19*) (inset). The numbers on the right of the inset denote apparent molecular mass in kilodaltons. Sperm were incubated for 5 minutes at 21°C (1.2×10^8 cells per milliliter) in Millipore-filtered seawater (*p*H 7.9), containing egg jelly in various dilutions, as above. At this sperm concentration, fucose at 1400 ng/ml was determined to be sufficient (on the basis of densitometric scans of silver-stained gels) to induce 99 to 100 percent conversion of the 160-kD form of the protein to 150 kD. The sperm were pelleted (12 minutes at 4000*g*); suspended in 0.25 percent Lubrol WX, 20 m*M* tris (*p*H 6.5), 10 m*M* NaF, and 100 μ*M* sodium vanadate (2°C); subjected to nitrogen cavitation for 10 minutes at 300 pounds per square inch (2°C); and assayed for guanylate cyclase activity. Samples were diluted so that product formation was linear with protein concentration (*32*) and time. Final assay conditions were: 0.06 percent Lubrol WX, 25 m*M* tris (*p*H 6.5), 1 m*M* 3-isobutyl-1-methylxanthine, 3 m*M* $MnCl_2$, 8 m*M* Na_3N, 20 m*M* NaF, 200 μ*M* sodium vanadate, 4 m*M* dithiothreitol, 1 m*M* cyclic GMP, 0.5 m*M* GTP (including [^{3}H]GTP at 10^6 cpm per 200-μl assay tube). All extracts showed similar recoveries of cyclic GMP (20 to 24 percent) and [^{3}H]GTP (90 to 100 percent) at the end of the assay. When extract A (specific activity: 91.5 nmol of cyclic GMP formed per minute per milligram) was mixed with an equal volume of extract E (2.28 nmol min^{-1} mg^{-1}), a specific activity of 48.2 nmol min^{-1} mg^{-1} was measured.

Arbacia punctulata sperm take up exogenous [^{32}P]phosphate and incorporate it predominantly into the 160-kD guanylate cyclase, in the form of [^{32}P]phosphoserine (*19*). Within the first 3 seconds after exposure of sperm to egg jelly all label is removed from the cyclase, and the mobility shift to 150 kD is observed (*19*). A similar mobility shift and loss of label from the 160-kD form of the enzyme can be induced in vitro with exogenous phosphatase preparations (*19*). Although partial proteolysis of the 160-kD form of the enzyme cannot be conclusively ruled out, we have suggested on the basis of these observations that the 160- to 150-kD mobility shift seen in vivo may be the result of a jelly-induced dephosphorylation of the 160-kD form of the enzyme. Such a large effect of phosphorylation state on electrophoretic mobility is not without precedent (*21, 22*) and is presumably due to an effect of charged phosphate groups on sodium dodecyl sulfate binding (*23, 24*). Egg jelly is known to induce an increase in protein phosphatase activity in sea urchin sperm (*16*).

In summary, we have demonstrated that the guanylate cyclase of sea urchin sperm is a phosphoprotein, that extracellular factors from the egg induce a change in its electrophoretic mobility (possibly as a result of dephosphorylation), and that correlated with this change in electrophoretic mobility is a change in guanylate cyclase activity. These results may help to elucidate the mechanisms by which extracellular factors from the egg activate the spermatozoan during fertilization.

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Evidence for Degenerative and Regenerative Changes in Neostriatal Spiny Neurons in Huntington's Disease

Abstract. *Golgi impregnations of neostriatum from deceased Huntington's disease patients and controls were examined. In all cases of Huntington's disease the morphology of dendrites of medium-sized spiny neurons was markedly altered by the appearance of recurved endings and appendages, a decrease or increase in the density of spines, and abnormalities in the size and shape of spines. Pathological changes were rarely observed in medium-sized and large aspiny neostriatal neurons. The findings provide evidence for simultaneous degeneration and growth of spiny neurons in Huntington's disease and support the view that a specific population of neostriatal neurons is selectively involved in its pathogenesis.*

Huntington's disease (HD) is a genetic disorder characterized in its late stages by severe motor and intellectual impairment (*1*). The cause of the disease is unknown. In neuropathologic studies of HD neuronal loss has been observed in many brain areas, including the basal ganglia and cerebral cortex (*2*). The caudate nucleus is thought to be the primary region affected because it consistently exhibits the most marked atrophy and cell loss. Studies have suggested that there may be a proportionally greater loss of small to medium-sized neurons than large cells in the neostriatum (*3*).

Golgi impregnations of the neostriatum in many species, including the human, reveal at least four types of neurons of small to medium size (*4*). The cell type impregnated most frequently is the medium-sized (diameter, 15 to 20 μm) spiny neuron, which has numerous dendritic spines and a long axon. It has been the best characterized of all neostriatal cell types in anatomical, immunohistochemical, and physiological studies (*5*).

In addition to being used in the study of normal cytoarchitecture, the Golgi method has been used in many brain