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Expression of DNA ligase genes by ram spermatid nuclei and RNA in amphibian eggs

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Summary. During animal development and gametogenesis two DNA ligases are found and successively expressed. In this study the two DNA ligases present in the axolotl egg and the two ligases present during ram sperm cell maturation were distinguished by biochemical and immunological methods. The expression of the genes for the heavy and light ram DNA ligases has been studied using transplantation of spermatid and sperm nuclei in axolotl eggs. We found that ram DNA ligases were expressed in axolotl egg cytoplasm. The exclusion phenomenon between the heavy and light form of DNA ligase is species-specific and involves a cytoplasmic mediator. In the transplanted ram germ cell nuclei the heavy ram DNA ligase expression was found to be sensitive to inhibitors of transcription while the light one was not. When mRNA was used, no exclusion process was observed and both the heavy and light enzyme expression were sensitive to cycloheximide and not to amanitin. These results are discussed in terms of the possible stability of the gene-regulated state following nuclear transfer.

Key words: DNA ligase – Gene activity – Nuclear transplantation – Ram spermatids – Axolotl egg

Introduction

Two different molecular forms of DNA ligase have been reported in various species, including mammals (Söderhäll and Lindahl 1975, 1976), chicken (David and Vinson 1979), amphibian (David et al. 1979) and sea urchin (Lefresne et al. 1984). The shift from an enzymatic form to another unrelated one is described as a peculiar developmental event. In axolotl, a 6S DNA ligase is present in the unfertilized egg and differentiated tissues, whereas an 8S form is observed in developmental stages (Carré et al. 1981). The replacement of the slow migrating (6S) by the fast migrating enzyme (8S) in early development has been proposed as a model for regulated changes in genetic activity (Signoret et al. 1981). Nuclear transplantation experiments have established that the specific enzymatic form of DNA ligase expressed by the injected nuclei is identical to the enzymatic form already present in the donor cell (Lefresne et al. 1983). This observation has been further supported by the use

of nucleocytoplasmic combinations of axolotl and *Pleurodeles* (Signoret et al. 1983). The isolation of messenger RNA (mRNA) for 8S DNA ligase and its cell-free translation have confirmed the involvement of specific structural genes in the process (Thiebaud et al. 1983).

In ram germ cells, a slow migrating form of DNA ligase (6.3S) is expressed in spermatocytes and in round spermatids. A faster migrating form (7.5S) is the only one expressed in elongated spermatids (David et al. 1982). The enzymatic shift occurs when spermatids elongate. Processes of spermiogenesis are unique among differentiating systems because diploid spermatocytes yield round haploid spermatids that become functionally anucleate while elongating due to chromatin condensation. In the present study, unfertilized eggs of axolotl were artificially activated and, in some experiments, ultra violet (UV) enucleated. Living nuclei of ram spermatids were prepared by cell lysis after separation of germ cells by centrifugal elutriation (Loir and Lanneau 1982). A suspension of nuclei was injected in individual recipient axolotl eggs and later, the material was analysed on sucrose gradients and tested for DNA ligase activity. The light and heavy forms of DNA ligase from ram and urodele differ in their sedimentation mobilities, allowing the molecular forms of each species to be distinguished. Additional characterization has been performed with antisera directed against axolotl 6S or axolotl 8S DNA ligase. Here we report that genes for DNA ligases from ram germ cells can be expressed in axolotl eggs and that the regulated state of this gene is retained in transplanted nuclei. Moreover, the use of RNA extracted from either round spermatid or spermatozoa resulted in the expression of stage-specific ram ligases when injected in axolotl eggs.

Materials and methods

Preparation of cells

Axolotl eggs (*Ambystoma mexicanum shaw*). These were obtained and sorted as previously described (Signoret et al. 1981), artificially activated by an electrical stimulation, and eventually UV-enucleated. Eggs were maintained at 20°C in spring water unless otherwise specified, until frozen in liquid nitrogen.

Ram cells. Ram germinal cells were separated by centrifugal elutriation according to the method described by Loir and Lanneau (1982). Immediately after castration, a suspension

of $1.2\text{--}1.9 \times 10^9$ testicular cells was prepared by trypsinization. After stopping the trypsinization by dilution with bovine serum albumin (BSA) at a final concentration of 0.5% and addition of an adequate quantity of lima bean trypsin inhibitor, the suspension was loaded at 10 ml/min in a Beckman elutriator driven by a J21B centrifuge at 4,000 rpm and kept at $18^\circ\text{--}19^\circ\text{C}$. By increasing the flow rate of the elutriation buffer (0.5% BSA in PBS + 3 mM potassium L-lactate) and decreasing the speed of the rotor, two germ cell populations (S, D) were collected. Depending upon their degree of maturation, spermatids were classified into form types: steps 1–8 (Clermont and Leblond 1955), round spermatids; and steps 13–15, elongated spermatids. The populations S and D contained respectively, as a mean, 99% elongated spermatids and 95% round spermatids.

Nuclear preparation and transplantation

For population D, the nuclei were prepared at $0\text{--}2^\circ\text{C}$ with 0.033% Triton X-100 in solution A (Tris-HCl 1 mM, pH 6.8, 0.25 M sucrose, 2 mM MgCl_2 , 1.2% BSA). After homogenization in a Potter-Elvehjem pestle fitted with Teflon (5–15 strokes) the suspension was centrifuged at 700 g for 10 min. The nuclear pellets were resuspended in solution A at a final concentration of 15–25 nuclei in 10 nl. For population S, the nuclei were prepared in the same way but with 0.080% Triton X-100 and the final nuclear concentration was adjusted to 45–60 nuclei in 10 nl.

We injected 10 nl of nuclei suspension into axolotl eggs following the procedure previously described (Signoret et al. 1983). When α -amanitin was used, unfertilized eggs were treated before activation for 24 h at 4°C with 200 $\mu\text{g}/\text{ml}$ of α -amanitin in Steinberg fluid (Signoret et al. 1981). Simultaneously, the ram germ cell nuclei were treated with 200 $\mu\text{g}/\text{ml}$ of the inhibitor in Steinberg fluid/solution A (1 vol./1 vol.) before being injected. The round germ cell nuclei were treated for 40 min at 2°C , while the steps 13–15 spermatid nuclei were treated for 1 h 20 min at 20°C .

Isolation of RNA and injection into eggs

RNA was extracted from 25.10^9 ram spermatozoa and $0.31.10^9$ round spermatids separated by elutriation. The two samples were separately processed for RNA extraction according to a modification of the method described by Chirgwin et al. (1979). Spermatozoa and round spermatids were homogenized in 7 ml of a solution containing 5 M guanidine thiocyanate, 1 mM EDTA, 2% sarcosyl and 0.1 M sodium acetate pH 5.6 at 20°C . Homogenates were centrifuged for 20 min at 10,000 g. Supernatants were layered on 1.2 ml of 5.7 M cesium chloride in 0.1 M EDTA, pH 7.4, and centrifuged for 20 h at 155,000 g in an SW₅₀ rotor. The RNA pellet was dissolved in sterile water at 4°C and precipitated three times in ethanol before being finally redissolved in sterile water. The RNA obtained 20 μg and 210 μg respectively for spermatozoa and for round spermatids) was checked for integrity on agarose electrophoresis (Mc Master and Carmichael 1977).

The injection of RNA into eggs was performed with a glass micropipette, which delivered 10 nl of fluid near the animal vegetative axis of the egg somewhat above the centre. No leakage was observed. Quantities of injected RNA per egg were 20 ng. Cycloheximide – treated eggs were activated in the presence of 10^{-3} M of the drug before

injection of RNA. The eggs were left in Steinberg's solution for 9 h then frozen in liquid nitrogen and used within a week.

Preparation of cellular extracts

We homogenized 20–40 eggs (approximately 0.1–0.5 g) at 0°C in 3 vol. of extraction buffer (0.5 M KCl, 20 mM Tris, 2 mM dithioerythritol and 0.2% NP40), made 0.8 mM in phenylmethylsulphonylfluoride (PMSF, Sigma) just before use. The cellular extract was sonicated (Alcatel Sonicator) for three periods of 15 s with 10-s intervals, at a dial setting of 2.2, and centrifuged at 140,000 g for 60 min at 4°C . The supernatant was used for sucrose gradient centrifugation.

Sedimentation on sucrose gradients

Aliquots of 250 μl were layered on 5%–30% sucrose gradients in 0.5 M CaCl₂, 50 mM Tris HCl, pH 7.4, 1 mM EDTA, 2 mM dithioerythritol and centrifuged for 15 h at 45,000 rpm in an SW50-1 rotor at 4°C . Fractions of 240 μl were collected and assayed for enzyme activities.

DNA ligase assay

DNA ligase was assayed according to a modification of the method described by Olivera (1971). The substrate was prepared as follows: 1 mM oligo dT_{12–18} (PL Biochemicals) in 50 mM Tris, pH 7.6, was incubated for 30 min at 37°C with 10 units/ml of *E. coli* alkaline phosphatase. The reaction was stopped by the addition of 0.1 vol. of 20 mM KH_2PO_4 . This mixture was adjusted to pH 10.5 with 1 N NaOH and kept for 15 min in a boiling water bath. After adjusting the pH to 7.6 with 1 N HCl, the extract was subjected to centrifugation at 10,000 rpm for 10 min. The supernatant, containing 200 μM of dephosphorylated oligo dT_{12–18}, was used for $5'$ - ^{32}P labelling in the presence of 60 mM Tris HCl, pH 7.6, 6 mM MgCl_2 , 6 mM 2-mercaptoethanol, 300 μM ATP (20 Ci-mmol) and 15 units/ml of T4-infected *E. coli* $5'$ -polynucleotide kinase (PL Biochemicals) at 37°C until the plateau of incorporation was reached. The resulting ($5'$ - ^{32}P) oligo dT_{12–18} was stored at -20°C until use.

The intensity and position of DNA ligase peaks on sucrose gradients were checked by the method of Modrich and Lehman (1970). For the determination of ligase activity, each assay (0.3 ml) contained 6 μM (^{32}P oligo dT_{12–18}, 6 mM poly (dA) (PL Biochemicals), 25 mM Tris HCl, pH 7.6, 6 mM MgCl_2 , 1 mM ATP, 2.5 mM dithiothreitol, 31 $\mu\text{g}/\text{ml}$ BSA and 100 μl enzyme solution. The samples were incubated at 37°C for 30 min. After the addition of 1 unit of alkaline phosphatase and dilution with 300 μl of ice-cold water they were incubated for 30 min at 80°C . The samples were precipitated with TCA, 5% final concentration, filtered through Millipore filters and counted. One unit of DNA ligase is defined as the activity which renders 1 nmol of ($5'$ - ^{32}P) oligo dT resistant to alkaline phosphatase in 30 min under standard conditions.

Immunological methods

The details for preparation and purification of antiserum will be published elsewhere (Raimbaud et al., in prepara-

tion). The IgG fraction was purified by gel filtration on a sephadex G200 column. Non-immune IgG was similarly purified from the preimmunized serum. IgG-containing fractions were stored in 0.1 M Tris HCl, pH 8.0 at -80°C . The antisera were used for enzyme neutralization assay after serial dilutions of the antibodies in 50 mM sodium phosphate, pH 7.6. The incubation of the enzyme fractions and serum protein was for 16 h at 4°C and the un-neutralized enzyme activity was assayed.

Results

Genes for ram DNA ligases are expressed in amphibian cytoplasm

The DNA ligase activities in axolotl eggs were distinguishable by sucrose gradient centrifugation. Extracts from activated non-enucleated and enucleated eggs showed a sedimentation coefficient of 8S and 6S respectively (Fig. 1A). The DNA ligase activities present in ram spermatids were also distinguishable with respective sedimentation coefficients of 6.3S and 7.5S (Fig. 1B).

Ten hours after injection of ram spermatid nuclei in axolotl eggs, the extracts were analysed for their DNA ligase activities. Recipient eggs were used either with or without enucleation. Eggs injected with ram spermatid nuclei present a peak of ligase activity typical of the recipient egg. Non-enucleated eggs activated and injected with nuclei from either round or elongated spermatids expressed the axolotl 8S ligase and either the 6.3S (Fig. 1C) or the 7.5S (Fig. 1D) ram ligase. Enucleated eggs, similarly injected expressed the axolotl 6S ligase and either the 6.3S (Fig. 1E) or the 7.5S (Fig. 1F) ram ligase. If activated non-enucleated axolotl eggs were injected with a mixture of nuclei from round and elongated spermatids, the 8S axolotl ligase was expressed together with both ram DNA ligases (Fig. 1G).

The identification of the axolotl and ram ligases was further checked using antisera against the 6S and the 8S enzymes from inactivated and activated eggs respectively (Raimbaud et al., in preparation). After purification of the antisera (see Methods) enzyme inhibition assays were performed using the anti-8S antiserum against the 6S axolotl and the 6.3S and 7.5S ram (Fig. 2A) enzymes and the anti 6S antiserum against the 8S axolotl, the 7.5S and 6.3S (Fig. 2B) ram enzymes. No cross-reactivity was observed. When the anti-8S antiserum was tested against the DNA ligase activities present in activated axolotl eggs injected with ram round spermatid nuclei, only one (6.3S) peak of activity was observed, thereby indicating the ram light DNA ligase (Fig. 2C). Repeating this experiment with activated axolotl eggs injected with nuclei from elongated spermatids, the 7.5S peak was positively identified as the ram heavy enzyme (Fig. 2D).

When enucleated axolotl eggs were implanted with nuclei from round and elongated spermatids and the extracts incubated with anti-6S antiserum, the 6.3S (Fig. 2E) and 7.5S (Fig. 2F) activities were found. Extracts from activated axolotl eggs injected with a mixture of round and elongated spermatids and treated with anti-8S antiserum present the 6.3S and 7.5S peak activities (Fig. 2G). These observations further establish the authenticity of axolotl and ram DNA ligase, and add to the evidence for expression of specific DNA ligase genes by foreign nuclei implanted in egg cytoplasm.

Fig. 1A-G. Sucrose gradient analysis of DNA ligase activity in extracts of axolotl eggs and ram spermatids. **A** Extracts from activated enucleated axolotl eggs ($\leftrightarrow$) and from activated non-enucleated axolotl egg ($---$). **B** Extracts from round spermatids ($\leftrightarrow$) and from elongated spermatids ($---$). **C** Extracts from activated axolotl eggs injected with nuclei from round spermatids. **D** Extracts from activated axolotl eggs injected with nuclei from elongated spermatids. **E** Extracts from activated enucleated axolotl eggs injected with nuclei from round spermatids. **F** Extracts from activated enucleated axolotl eggs injected with nuclei from elongated spermatids. **G** Extracts from activated axolotl eggs injected with a mixture of nuclei from round and elongated spermatids. A series of internal reference markers has been used including beef liver catalase (11.2S), chick DNA polymerase alpha (7.5S), *E. coli* alkaline phosphatase (6.3S), hen haemoglobin (4.2S) and horse heart cytochrome c (2.1S).

Fig. 2A-G. DNA ligase neutralization assay by specific antisera against the 8S and 6S axolotl enzyme. Before assaying the unneutralized enzyme activity, 10 μ l of the anti-axolotl 8S antiserum was preincubated at a 5-fold dilution with 100 μ l of each of the sucrose gradient fractions 3-7 of the extracts from elongated spermatids A; 10 μ l of the anti-axolotl 6S antiserum was preincubated at a 5-fold dilution with 100 μ l of each of the sucrose gradient fractions 9-13 of the extracts from round spermatids B. Under the same conditions anti-axolotl 8S antiserum was used on fractions 3-7 from extracts of activated axolotl eggs injected with either nuclei from round spermatids C or nuclei from elongated spermatids D. Anti-axolotl 6S antiserum was used on fractions 12-15 from extracts of enucleated activated axolotl eggs injected with either nuclei from round E or elongated F spermatids. Anti-axolotl DNA ligase 8S was used on fractions 1-15 from extracts of activated axolotl eggs injected with a mixture of nuclei from round and elongated spermatids G.

Exclusion process is species-specific

An interesting phenomenon already mentioned in amphibians (Signoret et al. 1983) is the reciprocal control of the two expressions of DNA ligase. The light axolotl enzyme is stable in enucleated eggs, but disappears within a few hours when the heavy enzyme is produced. Moreover, genes for the heavy and light forms of DNA ligase have never been found expressed simultaneously in the same species. However, the present experiment shows that the light form of DNA ligase from axolotl enucleated cytoplasm was not diminished in the presence of the heavy form of ram ligase controlled by elongated spermatid nuclei (Fig. 1F). Nevertheless, this 6S axolotl ligase vanished when the 8S type was expressed under the control of the female pronucleus in non-enucleated eggs, independently of the expression of the heavy type of ram ligase (Fig. 1D).

Similarly, the heavy axolotl ligase expressed in non-enucleated eggs did not interfere with the expression of the light ligase of ram produced after injection of ram round spermatid nuclei (Fig. 1C, 1D). When nuclei of round and elongated spermatids were mixed and injected simultaneously into axolotl eggs, the two forms of ram ligase were observed in addition to the axolotl enzyme (Fig. 1G). Thus the two molecular forms of DNA ligase of one species may be expressed simultaneously and co-exist, but only in a foreign cytoplasmic environment. The "exclusion process" fails to operate between nuclei of different species or between homospecific nuclei in heterospecific cytoplasm. Nevertheless, the process does work between the two non-allelic genes of the same nucleus.

Effects of inhibitors of transcription

The shift from a 6S to an 8S DNA ligase during axolotl development is sensitive to inhibitors of gene transcription (actinomycin D, amanitin Signoret et al. 1981). Eggs were injected with ram spermatid nuclei following treatment of both the nuclei and the eggs with amanitin. In all experiments, the expression of the 8S axolotl ligase was totally absent, while the 6S ligase was maintained undiminished. The two enzymes of axolotl and ram were present (Fig. 3A, dotted line) in the extracts of control activated axolotl eggs injected with round spermatid nuclei. When α -amanitin was used, the 6.3S ram ligase was expressed as in the absence of the inhibitor (Fig. 3A, solid line).

When the injected nuclei were from elongated spermatids, the enzyme activities observed in controls were heavy forms of axolotl and ram type (Fig. 3B, dotted line). However, when α -amanitin was used, the heavy form (7.5S) of ram ligase was absent (Fig. 3B, solid line). In this case, a light ligase (6.3S) was expressed and this enzyme activity was resistant to antiserum directed against 6S ligase from axolotl. This peak of activity was obviously assumed to be a light ligase of ram type. Thus the expression of the light form of enzyme by implanted nuclei appeared to be insensitive to inhibitors of transcription if already present in the donor cell. Moreover, this light DNA ligase is expressed as a consequence of such an inhibition for donor cells possessing the heavy form of enzyme and devoid of the light one.

Translation of injected ram RNA in axolotl eggs

As depicted in Fig. 4, the injection of RNA extracted from ram round spermatids in activated axolotl eggs resulted

Fig. 3A, B. Sucrose gradient analysis of DNA ligase activity in extracts of axolotl eggs injected with nuclei from either round A or elongated B spermatids in the absence (---) or presence of α -amanitin (—)

in the expression of a 6.3S enzyme together with the 8S enzyme normally present in activated axolotl eggs (Fig. 4A). The injection of RNA extracted from spermatozoa resulted in the expression of a 7.5S and the normally observed 8S enzyme (Fig. 4B). The injection of a mixture of equal amounts of RNA from round spermatids and spermatozoa resulted in the expression of three types of enzyme with sedimentation coefficients of 6.3, 7.5 and 8S (Fig. 4C). Pretreatment with cycloheximide abolished the expression of the 6.3S and 8S enzymes (Fig. 4D) in activated axolotl eggs injected with RNA from round spermatids. A control with antisera against axolotl 6S or 8S positively identified the enzymes from different origins. The expression of 6.3S and 7.5S DNA ligases in RNA-injected axolotl eggs was insensitive to α -amanitin (results not shown).

Discussion

The oocyte of Amphibia has been extensively used as a test system for expression of isolated DNA, RNA or whole nuclei (Gurdon and Melton 1981). Mature eggs have proved to be a convenient system for studying the expression of genes controlling the molecular forms of DNA ligases (Lefresne et al. 1983). Using UV-enucleation cytoplasm can be prepared with either one of the two enzymatic forms. On the other hand, transplanted nuclei can be from donor cells possessing either form of DNA ligase as a function of cell differentiation. The results reported in the present study are consistent with the stability of the regulated state of the genes for DNA ligase following nuclear transfer to egg cytoplasm. This assumption agrees with conclusions based upon intraspecific nuclear transplantations carried out using developmental stages of Amphibia (Signoret et al. 1984).

The expression of a gene by a nucleus transferred to a foreign cytoplasm raises the problem of the mechanism responsible for this expression. The possibility of a simple transfer of the terminal product can be ruled out because the intensity of the peak in the recipient egg corresponds

Fig. 4A–D. Sucrose gradient analysis of DNA ligase activity of activated axolotl eggs injected with RNA extracted from ram round spermatids A, ram spermatozoa B and a mixture of both C. D Ligase activity in activated axolotl eggs pretreated with cycloheximide and injected with RNA from round spermatids

to a much greater amount of material than the injected amount of substance, i.e. about 3 orders of magnitude.

Another possibility is the translation of transcripts carried over with the injected material into the recipient egg. Such a post-transcriptional expression is expected to be resistant to α -amanitin, and it could effectively support – at least in part – the expression of the light form of DNA ligase by ram round spermatid nuclei. Such nuclei have been shown to contain a significant amount of RNA (Loir 1972). Thus we consider that the nuclei from round spermatids are loaded with stable transcripts of the gene for the light form of DNA ligase. These transcripts could result from previous transcription and it is not known if they are renewed by continuing transcription (Loir 1972; D'Agostino et al. 1978; Erickson et al. 1980; Fujimoto and Erickson 1982). Corresponding messengers allow translation into the terminal product in spermatocytes and spermatids (light form of DNA ligase), as well as in transferred nuclei where there is an eventual inhibition of transcription.

The sensitivity of expression of the ram heavy form of DNA ligase to α -amanitin will not allow this mechanism in the case of elongated spermatid nuclei. These transferred nuclei are assumed to carry on a complete expression of the gene, through transcription, processing and translation. With respect to elongated spermatids in situ, although no precise data allow us to definitely exclude the possibility that the expression of the heavy DNA ligase form carried on in the elongated spermatids is an exceptionally late direct genetic activity, many results strongly support the complete absence of transcription due to tight packaging of the chromatin (Monesi 1964; Moore 1971; Loir 1972; Kierszenbaum and Tres 1975; Geremia et al. 1978; Loir and Courtens 1980; Balhorn 1982). In such a case, the heavy DNA ligase would be synthesized at this stage under a post-transcriptional positive control on transcripts and/or mRNAs previously produced. This has already been shown for another protein which appears in elongated spermatids simultaneously with the heavy DNA ligase, the protamine (Kleene et al. 1984). Such transcripts or mRNAs should be exclusively cytoplasmic and not introduced into the egg along with the nucleus. In elongated spermatid nuclei, transcripts for the light form of DNA ligase are still present although no longer expressed, probably as a consequence of the expression of the heavy form operating a post-transcriptional negative control. Stable RNAs may be inactivated in haploid cells by means other than degradation (Gold and Hecht 1981; Gold et al. 1983; Kleene et al. 1983, 1984; Stern et al. 1983). On the other hand, when in axolotl eggs, RNA from spermatozoa induced the synthesis of the heavy ligase form. Because the chromatin of elongated spermatids is very tightly packaged, we propose that the nuclei of these cells do not contain transcripts for the heavy ligase form but rather that mRNA coding for this protein is present in the cytoplasm. This form should result from the unmasking and processing of previously untranslatable transcripts in the intermediate spermatids, these cells being characterized by a massive transfer of the RNA from the nuclei to the cytoplasm. When elongated spermatid nuclei treated with α -amanitin are implanted in egg cytoplasm, these long-lived transcripts yield messengers able to support the expression of the light form of enzyme. Induction of enzyme production by transcription inhibitors has been reported in different systems (Tomkins et al. 1969, 1972). The process is suggested to involve long-lived transcripts and short-lived inhibitors of post-transcriptional expression.

The exclusion process controlling interaction between the two non-allelic genes has already been discussed (Signoret et al. 1983). The present report confirms the species-specificity of the mechanism: it fails to work between ram and axolotl, just as it fails between axolotl and *Pleurodeles*. Moreover, the exclusion process does not operate intraspecifically when different nuclei, controlling both enzymes, are in a foreign cytoplasmic environment. This result, already established with amphibian nuclei, is interpreted as indicating a species-specific cytoplasmic mediator in the process. However, as the process operates within the same nucleus even in a foreign cytoplasm, and as the light form seems to become unexpressed some time before the heavy form becomes present (David et al. 1982), we suggest that a multi-level system of reciprocal controls is responsible for the exclusion process.

At the present time, it is unknown what modifications

are induced by the cytoplasm of an amphibian egg in ram germ cell nuclei. Elongated spermatid nuclei possess a tightly condensed chromatin resistant to decondensation in the absence of a reduction of the -SS- bonds between protamine molecules (Loir and Lanneau 1978). Our results suggest that the gene for the heavy DNA ligase which is expressed in situ in elongated spermatids is in a non-repressed state. It would be interesting to know if some other genes, such as the protamine gene, which are also expressed in these cells are also expressed after transfer into axolotl eggs.

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