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Adenylate storage, metabolism and utilization in coelomic cells of the polychaete *Nereis virens* (Annelida, Polychaeta)

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Abstract. Eleocytes are specialized coelomic cells in nereid annelids which assume a central role during germ cell development. They may contain extremely high concentrations of both adenosine monophosphate (AMP) and adenosine diphosphate (ADP) (each >10 µmol/ml of cell vol.), whereas the adenosine triphosphate (ATP) content is comparatively low (0.8 µmol/ml cell vol.). ^{31}P nuclear magnetic resonance (NMR) studies of living eleocytes suggest the compartmentalization of both AMP and ADP in the large acidic vacuole characteristic for this cell type. Eleocytes are thus capable of storing high concentrations of ADP and AMP without inhibiting energy metabolism, by sequestering these compounds in a separate compartment. The high concentrations of both AMP and ADP in the eleocytes decrease in both males and females during the course of maturation. In eleocytes of male animals, the decline of the high nucleotide concentrations was accompanied by a transient increase of two intracellular nucleosides, inosine and guanosine. This suggests the degradation and further metabolism of nucleotides to the corresponding nucleosides. In culture, eleocytes release both inosine and guanosine into the medium. Both nucleosides are also present in the coelomic fluid, the common compartment for both eleocytes and germ cells. Both male and female germ cells incorporate ^{14}C-labelled inosine and guanosine in culture. For oocytes, the further incorporation of [^{14}C]inosine into the RNA fraction could be demonstrated. The large adenylate pools in the eleocytes may be regarded as a store for purine compounds for later use by the growing germ cells to supplement nucleic acid synthesis. The supply of nucleic acid precursors seems to be another specific function of eleocytes related to gametogenesis, in addition to their known synthesis of vitellogenin.

Key words. Polychaete; coelomic cells; eleocytes; adenylates; ADP; AMP; inosine.

Introduction

Nucleotides are the key metabolites of cellular energy metabolism, mediating the transfer of energy via cleavage and formation of phosphate bonds. They have a rapid turnover in the cell and usually do not reach exceedingly high concentrations in animal tissues. There are only a few cases in which the accumulation of large concentrations of nucleotides in animal tissues has been shown. High ATP concentrations (up to 100 mM) are found in the chromaffin granules of the medulla, where they may have a function in complexing the basic neurotransmitter acetylcholine[1]. In the embryos of the brine shrimp *Artemia*, large pools of diadenosine tetraphosphate are found, which serve as a source of purine compounds for the developing embryos[21].

In this contribution, we will present another example of nucleotide storage which we discovered recently in specialized coelomic cells in an invertebrate, the eleocytes of the polychaete *Nereis virens*. This cell type can contain high concentrations of both ADP and AMP. Before we focus on the role of these nucleotide stores, however, eleocytes and their known functions will be described briefly.

Characterization of eleocytes

Eleocytes are characterized by their large size (30–40 µm in diameter), a high lipid content (up to 30% of cell weight) and a large vacuole contributing 40–50% of the total cell volume[9,17]. Eleocytes are abundant in the coelomic cavity only during the reproductive phase of the life cycle, and a massive proliferation occurs shortly before the first gamete cells are formed, as was found for *Nereis virens* (Hoeger, unpubl. observation) and another polychaete, *Nicolea zostericola*[7]. In *Nereis virens*, the sexual maturation phase follows a seasonal time course covering a period of 20 months during which both male and female germ cells develop freely, floating in the coelomic compartment. Three main phases of gamete development can be distinguished in both sexes: an early phase of about 9 months, in which the gamete cells show little growth; an intermediate phase of about 4 months, in which the germ cells increase rapidly in biomass; and a late phase with no further growth in which the germ cells reach their final stage of development. In a population of maturing individuals, germ cell development is highly synchronized, and the animals die after spawning[9]. During the phase of germ cell development, eleocytes have specific

functions: they synthesize a yolk protein precursor (vitellogenin), the main component of the storage protein (vitellin) of the oocytes[8]. In addition, they phagocytose and digest muscle fragments which arise as a result of a characteristic reorganization of the body wall musculature during sexual maturation (epitokous metamorphosis)[6,9,10].

These observations show that eleocytes are well integrated into the processes of reproduction and gametogenesis in *Nereis virens*. In turn, the presence of large concentrations of AMP and ADP in these specialized cells raised the question whether the stored nucleotides could also have a function related to gametogenesis in this species. The experimental evidence that we have collected over the last five years to support this view will be discussed in the following section.

Nucleotide content of eleocytes

Metabolite studies on isolated eleocytes[14,15,20] revealed extremely high concentrations of both ADP and AMP each exceeding 15 µmol/ml of cell volume, while ATP was relatively low (0.6–0.8 µmol/ml cell vol). The maintenance of such high adenylate stores, however, poses a major problem for the cellular metabolism: if high levels of ADP and AMP are in free exchange with the cellular pools of ATP, an extremely low free energy of ATP hydrolysis will result and inhibit a multitude of enzymatic reactions involved in energy metabolism. ^{31}P NMR studies on living eleocytes[17] showed that the large pools of both ADP and AMP were free in solution and located in an acidic cellular compartment. Eleocytes contain a large vacuole characteristic for this cell type, and the acidic nature of this compartment could be easily demonstrated after in vivo staining with the pH-sensitive stain acridine orange[17]. The lysosomes form another possible acidic compartment, but they are unlikely to contain significant concentrations of nucleotides, owing to their content of hydrolytic enzymes. Therefore, the eleocyte vacuole is the most likely storage site for the large concentrations of ADP and AMP and thus can serve as a compartment to prevent the high nucleotide concentrations from interfering with cellular metabolism.

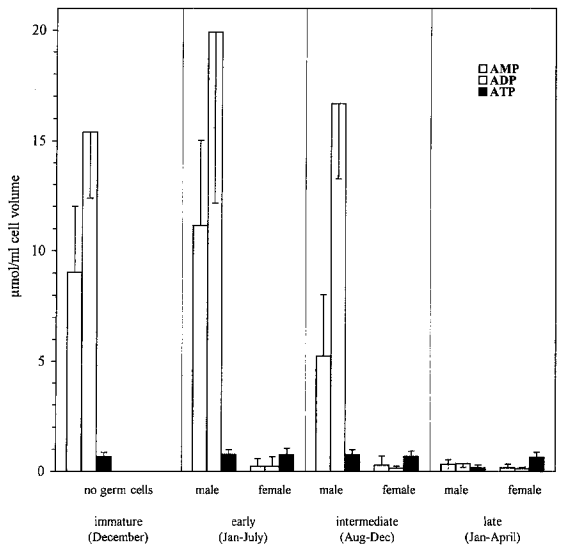

Figure 1. Adenine nucleotide content of eleocytes isolated at different stages of germ cell development. Bars represent mean values of 4–7 cell preparations; standard deviations are given. Data are taken from refs 14 and 20. Eleocytes were isolated according to ref. 13. For further characterization of the different stages of germ cell development, see figs 3a and 3b.

The high nucleotide concentrations were not always present, and were dependent on both the sex and the stage of germ cell development of the animal from which the eleocytes were isolated. Eleocytes of immature animals (i.e. animals before the formation of germ cells) always had high adenylate levels. These were maintained in eleocytes of males during the course of sexual maturation and decreased only at a late stage of gametogenesis. In contrast, eleocytes of females lost their adenylate stores soon after the formation of oocytes (fig. 1).

Degradation of nucleotides

The disappearance of the nucleotide stores during sexual maturation raised the question as to their further fate and led to studies of possible pathways and end products of degradation. Analysis of eleocyte extracts revealed the presence of several nucleosides, mainly inosine and guanosine, as well as lower amounts of the corresponding deoxynucleosides. In males, the highest inosine levels were present in eleocytes during the late phase, at a time when the AMP and ADP stores had already disappeared (fig. 2b). In contrast, when nucleotide levels were still high (intermediate phase; see fig. 1), rather low inosine levels were found (see fig. 2b). These inverse relationships hinted at a degradation of adenylate nucleotides mainly to inosine. In most tissues, inosine and other nucleosides are released under conditions of metabolic stress such as hypoxia. However, no end products of purine degradation such as uric acid, xanthine or hypoxanthine which are typically released under such conditions were found. This suggests that nucleotide degradation was not simply a disposal of waste nitrogen but that the purine nucleoside was saved for further utilization elsewhere.

The increase in the intracellular concentrations of inosine in male eleocytes (fig. 2b) did not match the decrease in the intracellular levels of both AMP and ADP (see fig. 1). Therefore, an obvious step was to study whether nucleosides could be released by the eleocytes into the coelomic fluid. In fact, when taken into culture, eleocytes were found to release inosine and

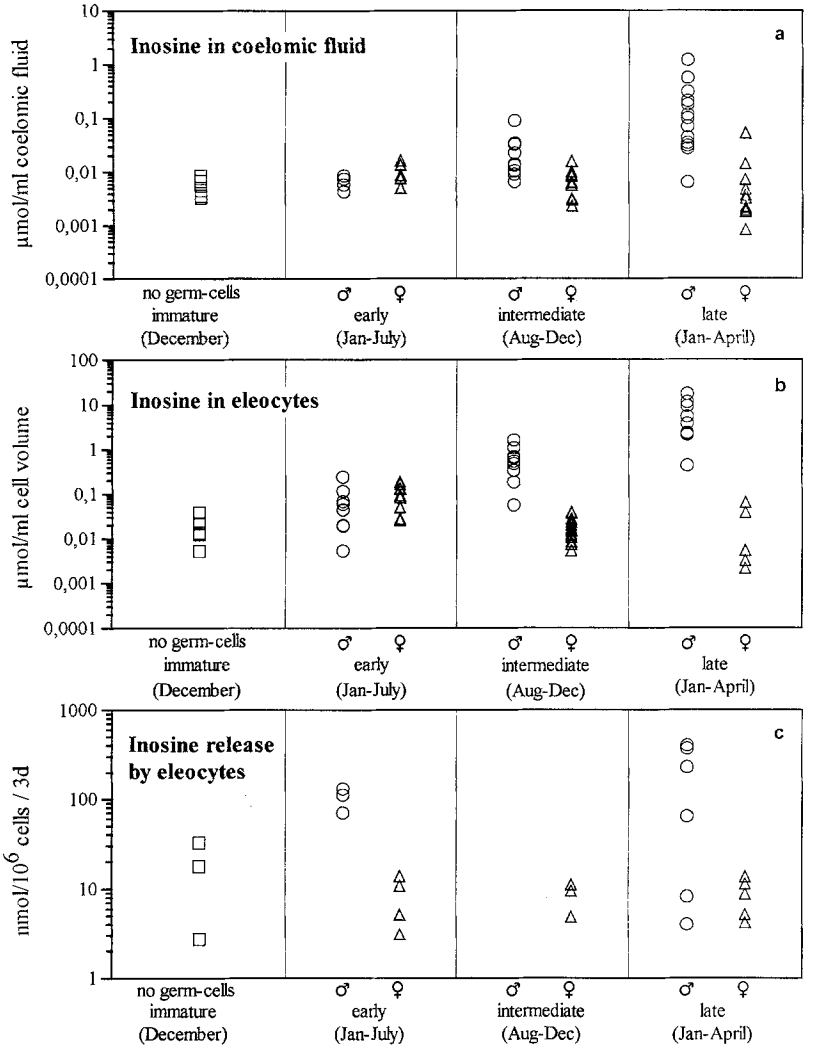

Figure 2. Comparison of the concentrations of inosine in the coelomic fluid (given as µmol/ml of coelomic fluid (*a*); and in eleocytes (given as µmol/ml of cell volume (*b*); with the rate of inosine release by eleocytes (given as nmol of inosine released into the medium per 10^6 cells in 3 days (*c*); at corresponding stages of germ cell development in immature (squares), male (circles) and female (triangles) individuals of *Nereis virens*. Data represent measurements obtained from individual samples of coelomic fluid and eleocytes, respectively. Coelomic fluid was obtained by puncturing the coelomic cavity of anesthetized animals with a glass capillary. Aliquots were heat-deproteinized and centrifuged, and the supernatant was assayed for nucleosides. Intracellular inosine concentrations (given as µmol/ml cell vol) were determined by HPLC in neutralized perchloric acid extracts of eleocytes (Hoeger and Märker, unpubl. data). Inosine release rates are taken from refs 10 and 11.

guanosine into the medium[10,11]. Again, sex- and stage-specific differences were evident (fig. 2c): while eleocytes from immature and female animals showed relatively low release rates of inosine, male eleocytes increased their release rates at the late stages of germ cell development, when their intracellular inosine content was high. Analysis of the coelomic fluid of *Nereis virens* at the corresponding maturation stages (fig. 2a) also revealed inosine and guanosine as the main nucleosides, and their levels were also characteristic for the stage of germ cell development, being highest in male eleocytes during the late phase of maturation (see fig. 2a). These observations were indicative of a nucleoside release by the eleocytes in vivo.

Fate of nucleosides

In subsequent studies, the fate of these nucleosides in the coelomic fluid was investigated. When incubated with ^{14}C-labelled inosine at concentrations resembling those in the coelomic fluid, both oocytes and male germ cells avidly incorporated the label[11,16]. Moreover, the uptake rates were correlated with the stage of germ cell development. In oocytes, up to six-fold higher uptake rates were found at the onset of the rapid growth (intermediate) phase compared with the early phase. The rates declined again during the late phase (fig. 3a). A similar situation was found for male germ cells (fig. 3b), which showed the highest uptake rates during the

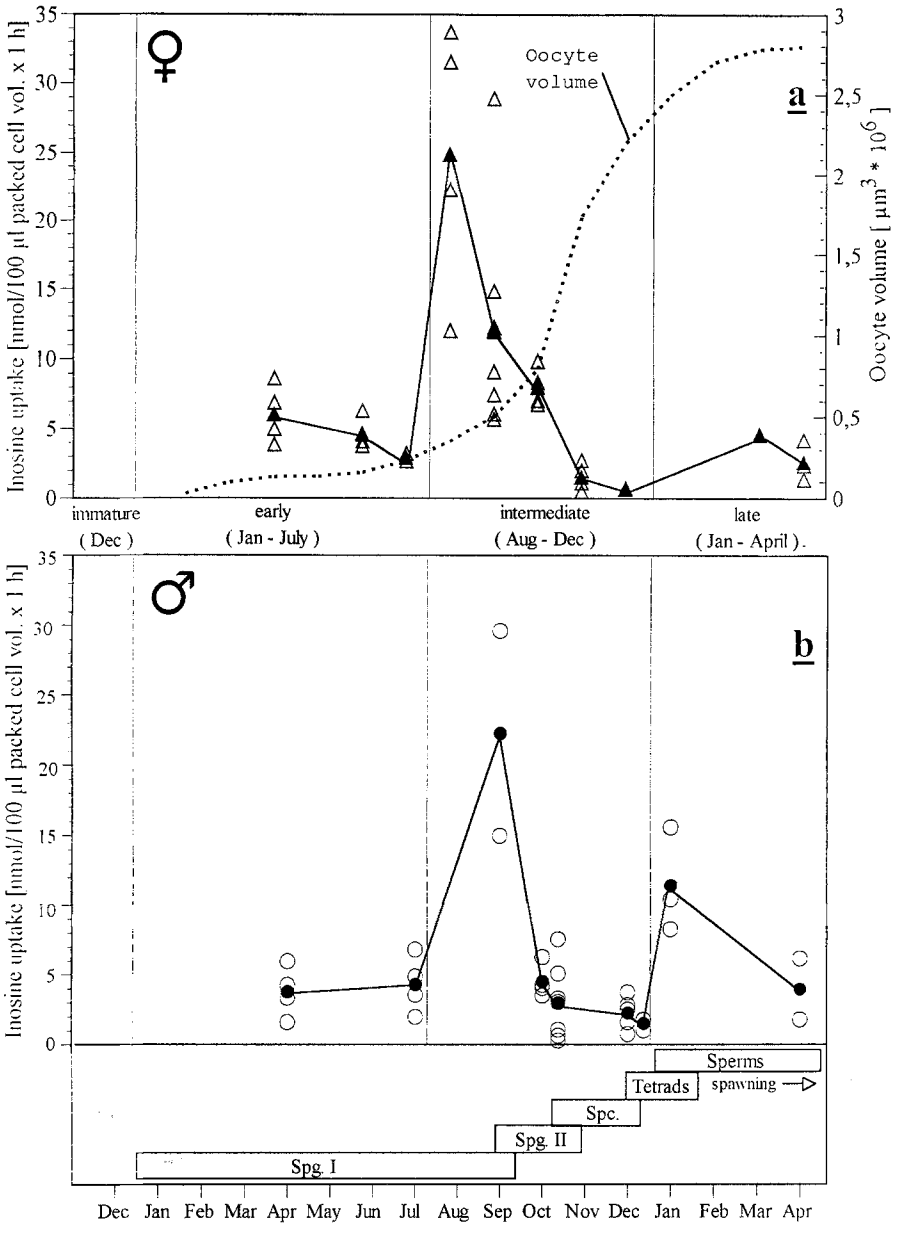

Figure 3. (*a*) Uptake of [^{14}C]inosine (given as nmol of inosine/100 µl of packed cell vol/h) by *Nereis virens* oocytes in different phases of development. Inosine concentration in the medium: 100 µM. Open triangles represent quadruplicate determinations obtained for oocyte preparations of individual animals; solid triangles denote mean values. The dotted line represents the increase in the mean oocyte volume (given as $\mu m^3 \cdot 10^6$) of the *Nereis virens* population investigated in our studies. (*b*) Uptake of [^{14}C]inosine (given as nmol of inosine/100 µl of packed cell vol/h) by different stages of male germ cells in *Nereis virens*. Inosine concentration in the medium: 100 µM. Data represent quadruplicate determinations on individual animals (open circles) and mean values (closed circles) are given as in fig. 3a. The bars in the lower part of fig. 3b denote the time period during which the different stages of male germ cells are present in a population of maturing individuals. The stages overlap slightly; more than one stage may be present in an individual. The time axis at the bottom of fig. 3b shows the period of 20 months during which the germ cell development in *Nereis virens* proceeds (see text). The data for oocyte volumes are combined from G. Geier (unpubl. data) and ref. 12. Classification of the male germ cell development stages are taken from ref. 19. Spg I, Spg II: spermatogonia stage I and stage II, respectively. Spc: spermatocyte stage.

transition from spermatogonial stage I to stage II, a phase which is characterized by high mitotic activities of the spermatogonia cells leading to a rapid increase in germ cell biomass. Interestingly, at a later stage when free spermatozoa develop in the coelomic cavity, male germ cells showed a second transient increase in inosine uptake. These characteristic changes in the uptake rates indicated an increased nucleoside demand at specific times during germ cell development which – in the case of the spermatogonia – corresponded to the phase of rapid biomass increase. The high uptake rates of the spermatogonia coincided with the rise in the intracellular concentrations of inosine in the eleocytes during this time (compare figs 2b and 3b), suggesting an increase in inosine supply for further export into the coelomic fluid (data for the corresponding inosine release rates

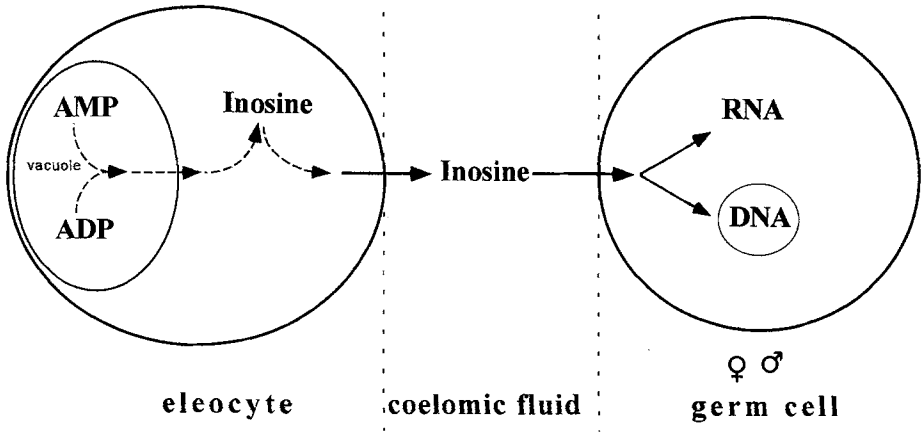

Figure 4. Proposed scheme of purine transfer from eleocytes to germ cells in *Nereis virens*. Solid arrows are supported by experimental evidence as discussed in the text. AMP and ADP are stored in the large vacuole of the eleocyte. Degradation of nucleotides leads to inosine as the main nucleoside, which is released by eleocytes into the coelomic fluid and taken up by both male and female germ cells, where it is incorporated into the nucleic acid fraction.

are not available at this time). In female eleocytes, this apparent coordination between nucleoside supply and demand was not evident. The decrease in the high levels of adenine nucleotides as the source for nucleosides occurred already during the early phase (see fig. 1), and both the release rate for inosine and its intracellular level remained far lower than in male eleocytes (see figs 2c and 2b).

An additional supply of exogenous precursors for nucleic acid synthesis seems very likely to be needed for the production of spermatozoa, which requires large amounts of DNA. For oocytes, exogenous nucleosides may seem less necessary, since oocytes increase only in volume but not in number during the rapid growth phase. However, nucleic acid precursors might nevertheless be important for oocytes. First, because the DNA content of *Nereis virens* oocytes exceeds that of somatic tissues by over 300-fold[23]. A similar situation is found in amphibian oocytes, where the high DNA content is the result of a large amplification of RNA coding genes[24]. Second, oocytes synthesize and store large amounts of ribosomal RNA. Labelling studies with [^{3}H] uridine in two other polychaete species, *Perinereis cultrifera* and *Schizobranchia insignis*, have indicated that RNA synthesis is most intense in small oocytes, i.e., at the beginning of oocyte growth[3,19]. The dramatic increase in the inosine uptake in *Nereis virens* oocytes seen during the rapid growth phase (see fig. 3a) is in agreement with these findings. In *Nereis virens*, our preliminary studies[11] showed that after incubation of oocytes with [^{14}C]inosine, part of the label was recovered in both the DNA and RNA fractions, suggesting that at least part of the exogenous inosine was utilized for nucleic acid synthesis. In future studies, we will compare the stage specific incorporation of exogenous nucleosides into the nucleic acid fractions with the corresponding rates of nucleoside uptake as discussed above.

Conclusion

Our present results on the significance of purine nucleotide stores in eleocytes of *Nereis virens* provide evidence for a transfer of stored purine compounds to the growing germ cells and their subsequent utilization for nucleic acid synthesis (fig. 4). Thus, in addition to their known function as suppliers of vitellogenin, the supply of purine compounds represents another specific function of eleocytes in the reproduction of *Nereis virens*. Stage- and sex-specific differences in both the time of nucleotide degradation and the rates of nucleoside release by the eleocytes, as well as the changes in the nucleoside uptake rates of the germ cells, suggest a regulation and coordination which could be mediated by hormonal factors. Unfortunately, our present knowledge on sexual maturation in nereid annelids is restricted to the presence of as yet unidentified hormonal factors, originating in the brain, which have been shown to affect various levels of gametogenesis including nucleic acid synthesis of the male and female germ cells[2,4,5].

An important point to be considered in future studies is the capabilities of germ cells to carry out their own purine biosynthesis. To evaluate correctly the significance of these exogenous purine stores, a comparison of exogenous purine utilization vs. endogenous biosynthesis is necessary. An additional point is that the inosine provided by catabolism of ADP and AMP stored in the eleocyte vacuole can provide only the purine building blocks for synthesis of nucleic acid precursors. As found for a variety of cell types, inosine can be trapped inside the cell by phosphorylation[22] after uptake and can provide both adenine and guanine nucleotides after enzymatic modification of the purine ring. However, if growth and development of the germ cells depend on exogenous nucleosides, a supply of pyrimidine nucleosides or bases would also be necessary. Eleocytes,

however, were not found to release significant amounts of pyrimidine nucleosides into the medium in culture.[10] In contrast, the uptake of uridine and its subsequent incorporation into the nucleic acid fraction has already been shown for both male and female germ cells of polychaetes[3,4,5,19]. Therefore, pyrimidine precursors would have to be generated by autosynthesis inside the germ cells or supplied from tissues other than eleocytes. The answer to the question of why eleocytes store purine compounds in the form of nucleotides remains a matter of speculation at present. Nucleotides carry a strong charge, owing to their phosphate bonds, and this might prevent them from diffusing through the vacuole membrane.

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