ATP-Content in Unfertilized and Fertilized Eggs of *Ciona intestinalis*

A marked change in shape occurs in the ascidian egg at fertilization; the egg cortex undergoes movements from the animal to the vegetal pole and the ooplasmic components redistribute and segregate in five different regions[1-3]. Mitochondria that in the unfertilized egg form a thick layer at the periphery of the egg, except in a limited region of the animal pole, in the fertilized egg collect almost entirely at the vegetal pole[4,5]. The presence of a contractile protein in the egg cortex, which might be activated at fertilization and could be responsible for the modifications reported above, agrees with the results obtained on the activity of the actomyosin-like ATPase in both unfertilized and fertilized eggs of *Ciona intestinalis*[6]. In fact, while no activity is present in the unfertilized egg, enzyme activity is detected after fertilization.

Metabolic changes are associated with the morphological variations discussed above, but there are as yet few data on this aspect of the problem. A noticeable increase in oxygen consumption has been detected by many authors[7-11], but this fact is not very indicative: in fact it is more important to know how this change is brought about. The best way to investigate this problem seems to be a study of the activity of enzymes connected with oxidative metabolism. An analysis of cytochrome oxidase activity has been reported in a previous work; an increase of 25% in enzyme activity in the fertilized egg has been attributed either to an inhibitor release or to a change in the permeability of mitochondrial membrane[12].

Since ATP is the end product of oxidative metabolism in the cell, an analysis on the content of this substance in unfertilized and fertilized eggs of *C. intestinalis*, has been carried out in order to elucidate the mechanism which regulates respiration of the ascidian egg at fertilization.

Materials and methods. The samples for measurement of the ATP concentration were prepared in the following manner: The eggs were removed directly from the oviduct of a single animal, washed with artificial sea water[13], collected in aliquots of 1000 and placed in small containers; fertilization was carried out by adding a very diluted drop of sperm suspension; 5 min later, after being scrupulously washed, the eggs were transferred into a homogenizer. Braking pipettes were used for the transfer in order to collect the eggs in a small volume. Homogenization was easily done in an ice-cold, all-glass homogenizer after addition of 0.5 ml of double glass-distilled water, the homogenizer was rinsed with the same volume of distilled water and the homogenate plus rinse were centrifuged in the cold after addition of enough cold perchloric acid to have a final concentration of 4%. The clear supernatant was neutralized in the cold with KOH. The potassium perchlorate was removed by centrifugation. The neutral solution combined with a perchlorate wash was then vacuum-dried in the cold. Finally the dry extract was dissolved in 1 ml double distilled water and parts of this solution were used for the assay.

ATP content in the samples was measured by the method of STREHLER and MCELROY[14]; the crude extract of firefly lanterns was applied as the luciferin-luciferase system. Luminescence was measured with a liquid scintillation counter manufactured by Nuclear Enterprise Edinburgh.

The reaction mixture which had a final volume of 10 ml was composed of sodium arsenate buffer 0.01 *M* pH 7.4; 4 μM $MgSO_4$ $7H_2O$ and firefly extract equal to 0.7 mg of dry lantern. The luminiscence was measured for 1 min beginning 30 sec after ATP addition.

Chemicals. ATP disodium salt and firefly desiccated abdomens were obtained from Sigma Chem. Co., USA; sodium arsenate, magnesium sulphate, potassium hydroxyde and perchloric acid were supplied by Merck, Darmstadt (Germany).

Results. The Table summarizes the data obtained from a series of experiments carried out on eggs of single animals; preparation of extracts and measurement of the ATP content were made contemporaneously both in unfertilized and in fertilized eggs; each value is the mean of 3 assays on the same extract. Preliminary experiments were carried out in order to keep the ATP concentration within those limits which, for a given amount of enzyme, gave a linear distribution of the counting rate. Those limits were fixed between 0.1 µg and 0.01 µg ATP, in any case a standard was run for each measurement. Data for fertilized eggs refer to extracts prepared 10 min after fertilization. A tremendous decrease of ATP level after fertilization is clearly shown, although slight variations are observed from one experiment to another. The ratio between the ATP content in unfertilized and fertilized eggs is constant.

Discussion. From the results obtained it is evident that the unfertilized egg of *C. intestinalis* contains a reserve of ATP, probably accumulated during oocyte maturation, by means of which the energy requirements following fertilization can be satisfied. A sevenfold decrease of ATP level occurs in the egg at fertilization; this

ATP-content in eggs of *Ciona intestinalis*

Experiment No.	Unfertilized	Fertilized	U/F ratio
1	6.56	0.99	6.57
2	8.37	0.79	10.50
3	8.48	1.10	7.70
4	10.85	1.70	6.40
5	5.20	0.68	7.65
6	7.58	1.02	7.44
7	12.44	1.81	6.90
8	5.53	–	–
9	11.08	1.58	7.02
Means	8.43	1.21	7.52
S.E.	± 0.85	± 0.05	± 0.46

Data expressed in $\mu g \cdot 10^{-4}$/egg.

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fact is an expression of the large amount of work which is done by the egg. The question is how ATP is utilized. Contractile processes on the egg cortex, connected with the actomyosin-like ATPase activity, bring about considerable changes in the shape of the egg and movements of the cortex toward the vegetal pole[1,2,6]; the ooplasmic components are mixed up and segregated into 5 different regions[3], while mitochondria collect at the vegetal pole[4,5]. All those modifications require energy expense which is supplied by the ATP present in the unfertilized egg.

Moreover the role of ATP in amino acid activation for the protein synthesis must be taken into account; a noticeable increase of activated amino acids has been found in the egg of *C. intestinalis* after fertilization[15]. The rise in permeability of the egg membrane could also be responsible for ATP consumption, since ATPase present in cell membrane plays a role in the active transport of sodium and potassium[16].

ATP is closely linked to all the phosphorilating processes in the cell; it has been reported that in sea urchin eggs after fertilization there is a marked increase in some of the phosphorilated substrates of glycolisis[17]; moreover a noticeable increase of NADP has been detected at the expense of NAD[18]. An investigation of the activity of phosphorilating processes in the ascidian egg is now in progress in our laboratory.

If we compare the oxygen consumption with the ATP level in unfertilized and fertilized eggs, it is clear that in the former a low level of oxygen consumption corresponds to a high ATP content, while in the latter high oxygen consumption is accompanied by a low ATP level. It seems, therefore, that the release of respiratory control following fertilization is related to ATP consumption. In the sea urchin egg such a situation has been found: a noticeable ATP decrease occurs at fertilization[19,20].

An analysis of ADP level revealed a low concentration in the unfertilized egg and a high level in the fertilized egg[20]. Since it is known that the concentration of high energy phosphate acceptors is a regulatory factor in respiration[21,22], the low level of ADP could, according to the authors, be responsible for the low level of oxygen consumption of the unfertilized egg. Measurements of ADP content in unfertilized and fertilized eggs of ascidians which are in progress in our laboratory could throw light on this problem.

Riassunto. Il dosaggio dell'ATP presente nelle uova è stato fatto col sistema luciferin-luciferasi misurando la luminescenza mediante un contatore a scintillazione liquida. È stato rilevato che 10 min dopo la fecondazione il livello di ATP nelle uova si abbassa di ben sette volte. Il risultato è discusso sulla base delle modificazioni morfologiche e biochimiche che intervengono nell'uovo alla fecondazione.

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Differentiation of Haustoria in the Germinating Embryos of Mistletoe Without Host Stimulus

In recent years morphogenesis of embryo of parasitic angiosperms has attracted much attention[1–4]. The seedlings of parasites raised in cultures, in the absence of the host, usually fail to differentiate haustoria. This communication describes in vitro differentiation of haustoria from the excised embryo and endosperm of *Scurrula pulverulenta*, in the absence of host tissue or host extract.

Mature fruits of the parasite were surface-sterilised with absolute ethyl alcohol for 10 min. The 'seeds' (lacking a seed coat; Figure A) were excised and planted on modified White's semi-solid medium (WM) without IAA[5] containing 2% sucrose. In some treatments WM was supplemented with casein hydrolysate (CH), watermelon juice (WMJ), IAA, and cytokinins, individually and in various combinations.

On White's semi-solid medium the seeds germinated in 45% of the cultures. Although the addition of 400 ppm CH did not improve the percentage of germination, the seedlings appeared robust (Figure B). On WM as well as WM + CH, 46% seedlings formed haustoria. The addition of 10^{-5} *M* benzyladenine (BA) to WM increased the percentage of germination to 90; and 60% of the seedlings developed haustoria. The percentage of germination on WM + 10% WMJ was comparable to that on WM + BA, but the seedlings on the former medium failed to form haustoria. The differentiation of haustoria was also inhibited by coconut milk.

In 45% of the cultures raised on WM + 400 ppm CH + 1 ppm IAA, after 5 weeks the embryo proliferated into a callus upon coming in contact with the nutrient medium. The resulting callus was compact, green and slow-growing. Several tracheid-like cells were observed in a 14-week-old callus. After 20 weeks the callus bore shoots, or haustoria, or both (Figure C).

Proliferation of the embryo also occurred if 1 ppm kinetin was added to WM + CH + IAA. However, the callus was friable, non-chlorophyllous, and either a shoot or a haustorium failed to differentiate.

On WM supplemented with BA, kinetin, 6-(γ,γ-dimethylallylamino)-purine, or zeatin, each at 10^{-5} *M*, shoot buds differentiated from the peripheral cells of the un-

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