

TRANSCRIPTIONAL REGULATION DURING THE ACTIVATION AND DEVELOPMENT OF *Artemia franciscana* ENCYSTED EMBRYOS

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Early embryos of the crustacean *Artemia franciscana* (brine shrimp) can get into cryptobiosis under adverse environmental conditions. Under these circumstances, the embryo arrests all metabolic activities, gets dehydrated and is surrounded by a hard shell. These cysts are viable for long periods and can be activated when environmental conditions are favorable. Once the cyst is activated, the embryo resumes development and gives rise in a few hours to a swimming nauplii that continues the development, through several molts, to the adult animal. The activation of the cyst can be reproduced in the laboratory which makes of the *Artemia* cyst an useful model system to study the mechanisms that regulate the exit of a latent state, as well as embryonic development. Activation of the cyst requires resumption of all metabolic activities, including energetic metabolism, protein synthesis, gene transcription, etc. While the mechanisms involved in the activation of the metabolism and protein synthesis have been studied with some detail in the last years (1), little is known about the process of transcriptional activation. The only well established data is the presence of active RNA polymerases in the cyst which implies that the cysts have the enzymatic activity required for transcription (2). These data suggest that the lack of transcriptional activity in the cyst must be regulated through the accessibility of the RNA polymerases to the gene promoters. Our laboratory is trying to test this hypothesis and to get more information on the mechanisms that regulate gene transcription during the activation of the cyst.

In the last years we have isolated cDNA and genomic clones coding for several genes whose expression is induced during the embryonic development that immediately follows cyst activation. These genes encode the Sarco/Endoplasmic reticulum Ca-ATPase (3), the $\alpha 1$ subunit of the Na/K ATPase (4) and three actin isoforms (5). The mRNAs encoded by all these genes, except for one actin isoform, are expressed in the cyst but their levels are markedly increased during the first hours of embryonic development (6). The mRNA encoded by the Actin 302 gene is not expressed in the cyst and is induced after 10 hours of development (6). These studies show that gene transcription is activated in the first hours of development, after the activation of the cyst.

Once the expression pattern of these genes had been characterized, we have approached the study of the mechanism involved in the regulation of their expression through the isolation and characterization of their promoter regions. The exon/intron structure of these genes was established and their transcription initiation sites determined (3-5). The sarco/endoplasmic reticulum Ca-ATPase gene was exceptional since this gene can be transcribed from two different promoters to give two mRNAs of different size that code for two protein isoforms differing at their C-terminal amino acids and that are expressed in different tissues (7,8).

Promoter	Cell line		
	NIH3T3	N ₂ A	C ₂ C ₁₂
$\alpha 1$, Na/K ATPase	8.6±2.1	4.1±3.3	-
SERCA-1	5.4±3.7	16.4±6.2	9.3±4.9
SERCA-2	38.3±28.6	-	19.6±4.1
Actin 211	1.8±0.6	3.0±1.1	1.9±0.4
Actin 302	8.3±3.3	53.0±16.1	9.5±3.8
Actin 403	16.6±3.4	9.2±1.0	166.0±4.7

Table I. Relative luciferase activity expressed under the control of the *Artemia franciscana* gene promoters. Luciferase activity is expressed as folds of induction over the activity of the reporter gene vector without any promoter region. NIH 3T3 is a mouse embryo fibroblastoid cell line, N₂A a mouse neuroblastoma and C₂C₁₂ a mouse myoblast. SERCA: Sarco/Endoplasmic Reticulum Ca-ATPase.

The isolation of the different promoter regions has allowed the analyses of the putative transcription factors binding to them in cryptobiotic cysts and in developing nauplii. DNase I footprinting assays have shown the presence in nuclear extracts from nauplii of proteins able to protect rather extensive areas of the promoters, including the transcriptional initiation site and immediately upstream regions. However, nuclear extracts from cysts hardly produce any protection on the promoter regions. These results suggest that transcription initiation complexes are being assembled when the promoter regions are incubated with nauplii nuclear extracts but not when incubated with cysts nuclear extracts. These results could be explained either by the absence of some transcription factors in the cysts extracts or by the presence of some inhibitory activity. The mixture of nuclear extracts from cysts and nauplii produce the same protection as naupliar extracts alone, suggesting that cyst extracts do not contain inhibitors of transcription factors binding.

The second hypothesis, the absence of transcription factors from the cyst is being tested in more detail. One of the possibilities would be the absence of general transcription factors. We have studied the basic transcription factor TBP (TATA Binding Protein) since it is involved in the formation of the initiation complex by the three RNA polymerases. *Artemia franciscana* TBP gene is composed of seven exons and codes for a protein highly homologous to TBP from other species in the conserved C-terminal region. The analyses of the expression of this gene has shown the both TBP mRNA and protein are present in the cyst.

Other important group of transcription factors are those that are sequence specific, recognizing regulatory elements in promoter and/or enhancer regions. The identification of regulatory elements in the *Artemia franciscana* promoters is being approached by transient expression experiments using construct that express the luciferase gene under the control of the *Artemia* promoters. The lack of *Artemia* cultured cell lines compelled us to use mammalian cell lines in these experiments. All the *Artemia* promoters that have been studied induce luciferase activity in several mammalian cell lines that have been tried (Table I) which indicates the evolutive conservation of some of the transcriptional regulatory elements between crustaceans and mammals. At the present time, we are analyzing the promoter activity of progressive deletions of the *Artemia* promoters to identify their more important regulatory elements. Alternatively, possible regulatory elements have also been identified by DNase I protection experiments using *Artemia* nauplii nuclear extracts. Oligonucleotides representing some of the protected regions have been used in mobility shift assays to study the possible proteins binding to these regions. These experiments have shown the formation of specific oligonucleotide-protein complexes when nauplii nuclear extracts are tested but not when cysts nuclear extracts are used. Similar to what was found in DNase I protection assays, the presence of cyst nuclear extracts do not interfere with the interaction of the nauplii nuclear proteins with the oligonucleotides. These results suggest that the promoter binding proteins that have been studied are not present in the cyst and are induced after their activation. The extension of these tests to other transcription factors, especially those binding to the elements of the promoters found to be important in the transfection assays, would be of great interest and is being approached in our laboratory at the present time.

References

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