3-Hydroxy-3-methylglutaryl Coenzyme A Reductase in the Sea Urchin Embryo Is Developmentally Regulated*

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The activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, an enzyme which plays a regulatory role in the synthesis of cholesterol, dolichol, and coenzyme Q, has been measured in the developing embryo of the sea urchin. Enzyme activity increased at least 200-fold during development from the unfertilized egg to the pluteus stage embryo. Mixing experiments suggested that the low level of enzyme activity found at early stages was not due to the presence of inhibitor(s) in the egg or zygote. The enzyme in the sea urchin embryo exhibited properties different from that found in mammals: only a fraction of the activity could be solubilized from microsomes, and mild tryptic inactivation inactivated the enzyme without releasing any of it from the microsomes in soluble form. To further study the sea urchin HMG-CoA reductase, a genomic clone was identified by hybridization to a cDNA encoding hamster HMG-CoA reductase. Sequence analysis of this clone revealed a coding region that shares a high degree of homology with the carboxyl-terminal domain of hamster HMG-CoA reductase. Analysis of sea urchin embryo HMG-CoA reductase mRNA levels using a restriction fragment derived from the genomic clone revealed a 5.5-kilobase poly(A)* mRNA that increased 15-fold during development from the egg to the gastrula stage and then decreased 1.5-fold at the pluteus stage. Since the relative increase in HMG-CoA reductase mRNA was less than the increase in enzyme activity (15-fold versus 200-fold) factors in addition to the level of mRNA may control the activity of this enzyme during embryogenesis.

Mammalian 3-hydroxy-3-methylglutaryl-CoA(HMG-CoA)* reductase is a 97-kDa transmembrane glycoprotein of the endoplasmic reticulum (1, 2). The nucleotide sequences of cloned cDNAs for human and hamster HMG-CoA reductase have been determined and the amino acid sequences deduced (3-5). In addition, the 25-kb hamster HMG-CoA reductase gene has been identified and shown to contain 20 exons (6).

Recently, two HMG-CoA reductase genes from yeast have been identified and shown to share 64% amino acid homology with the hamster reductase cDNA (7).

These studies, coupled with biochemical experiments, have provided a detailed picture of the structure of this membrane-bound enzyme. The mammalian enzyme can be proteolytically cleaved into two domains, an amino-terminal, membrane-bound domain of 339 residues and a carboxyl-terminal, soluble domain of 548 residues projecting into the cytosol and containing the catalytic site (2). This catalytically active fragment can be released in soluble form from the endoplasmic reticulum by either endogenous or exogenous proteases (2, 8). The single carbohydrate attachment site is located in the amino-terminal domain of the molecule and is oriented toward the lumen of the endoplasmic reticulum (2, 3). Modeling studies of the deduced primary structure of HMG-CoA reductase suggest that the amino-terminal domain contains seven distinct membrane-spanning regions (2).

In mammalian cells, HMG-CoA reductase is the rate controlling enzyme of polyisoprenoid biosynthesis (9) and provides the precursor, mevalonial acid, common to cholesterol, dolichol, and coenzyme Q. Studies in this laboratory have shown that de novo synthesis of N-linked glycoproteins is required for normal gastrulation in the sea urchin embryo (10). Furthermore, it has been established that dolichol, in the form of dolichylphosphate, is of particular importance as a biosynthetic intermediate during sea urchin embryogenesis because it functions as a carrier of oligosaccharide chains that ultimately are N-linked to proteins (11, 12). Compactin, a competitive inhibitor of HMG-CoA reductase, inhibits dolichol and cholesterol synthesis in the developing embryo. The consequence of this inhibition is a block in glycoprotein synthesis and a concomitant lack of normal gastrulation (11, 12). This compactin-induced inhibition of normal gastrulation can be prevented by culturing embryos in the presence of exogenous dolichol or dolichylphosphate, but not cholesterol or ubiquinone, suggesting that de novo synthesis occurs prior to gastrulation and that it is a prerequisite for glycoprotein synthesis and normal development. In agreement with this idea, polyisoprenoid biosynthesis has been shown to increase dramatically following fertilization (12).

These findings suggest that the regulation of HMG-CoA reductase activity has direct consequences on early sea urchin embryonic development. For this reason, in the present study we have partially characterized sea urchin embryo HMG-CoA reductase and measured its activity over the course of development. In addition, we have isolated and sequenced a fragment of the sea urchin embryo HMG-CoA reductase gene and studied some aspects of the regulation of this enzyme. Although biochemical studies reveal that the properties of this enzyme differ in several respects from the mammalian enzyme, sequence analysis indicates that the active site in the
cytoplasmic domain is highly conserved. The results of a comparison of changes in enzyme activity and mRNA levels over the course of development suggest that factors in addition to mRNA availability may control the level of HMG-CoA reductase activity.

EXPERIMENTAL PROCEDURES AND RESULTS

The present study demonstrates that HMG-CoA reductase activity is developmentally regulated during sea urchin embryogenesis. The increase in enzyme activity (200-fold) during development parallels that observed for both de novo polyisoprenoid biosynthesis and glycoprotein biosynthesis in the sea urchin (10, 12), suggesting coordinate expression of HMG-CoA reductase activity, dolichol synthesis, and glycoprotein biosynthesis during embryonic sea urchin development.

To elucidate the properties of the sea urchin embryo reductase, several approaches were taken to obtain a soluble form of the enzyme. The results indicate that sea urchin embryo HMG-CoA reductase differs from the mammalian enzyme with respect to several physical properties. The sea urchin enzyme cannot be readily solubilized from microsomes by glycerol/freeze-thawing or extraction with KCl treatments which are known to release enzyme activity in soluble form from mammalian microsomes (2, 8). The absence of an endogenous protease(s) in the sea urchin embryo that releases soluble enzyme activity may account for the lack of solubilization by these reagents. However, it seems more likely that the appropriate cleavage sites in the sea urchin reductase are absent because an alternative approach using trypsin treatment, to activate enzyme was also unsuccessful. The results of these experiments indicate that, unlike the mammalian enzyme, the sea urchin embryo enzyme is inactive faster than it can be solubilized from the microsomes. Another difference between the sea urchin embryo enzyme and the mammalian reductase is that a variety of detergents solubilize only 20–30% of the sea urchin enzyme. This is in contrast to studies that have shown quantitative solubilization of enzyme activity from UT-1 cells with Triton X-100 and deoxycholate detergents (1). This difference between the mammalian enzyme and the sea urchin embryo enzyme could be due to structural differences in the membrane-spanning domains or in compositional differences in the lipids of the microsomal membranes.

Based on the above observations it became clear that information about the structure of the sea urchin embryonic enzyme might be more readily obtained using recombinant DNA techniques. Initially, three different sea urchin embryo cDNA libraries were unsuccessfully screened with a 2.2-kb hamster HMG-CoA reductase cDNA. Therefore, a sea urchin genomic library was screened with this same cDNA probe, which encodes a portion of the cytoplasmic domain of the enzyme that is known to contain the active site and to be conserved in humans (4), rodents (3), and yeast (7). One genomic clone, XG1, was identified as containing part of the gene for sea urchin embryo HMG-CoA reductase. DNA sequence analysis of the region of cross-hybridization indicated that the predicted amino acid sequence of the protein encoded by this sea urchin embryo gene shared a high degree of homology (78%) with the sequence of a region (residues 672–718) in the carboxyl-terminal part of the hamster HMG-CoA reductase protein. Human (4) and yeast (7) HMG-CoA reductase DNAs have been found to share similar high homology in this domain with each other and with the hamster reductase. Our findings in this study support these earlier studies and suggest that this region of HMG-CoA reductase containing the active site has been conserved in species that are phylogenetically far apart.

Sequence analysis of XG1 revealed other features of interest. The 0.43-kb PstI-AvaII restriction fragment, which cross-hybridizes with the heterologous rodent cDNA, contained both coding and noncoding sequences. The coding sequence, which comprises one-third of the total sequence of this fragment, hybridized exclusively with the 5.5-kb sea urchin embryo HMG-CoA reductase mRNA. The noncoding portion of this fragment hybridized with minor 4.0-, 3.5-, and 2.5-kb mRNA species, but not with the 5.5-kb reductase mRNA, indicating that this region of DNA shared no homology to sea urchin embryo HMG-CoA reductase. Preliminary experiments using restriction fragments derived from the 5' end of the hamster HMG-CoA reductase cDNA suggest that XG1 does not contain the 5' end of the sea urchin HMG-CoA reductase gene (data not shown). However, a second genomic clone, XG4, isolated from screening the genomic library, cross-hybridizes with these 5' hamster HMG-CoA reductase fragments; this clone may share DNA sequence homology with the 5' domain of HMG-CoA reductase. We are currently isolating and characterizing additional 5'-overlapping genomic clones in order to delineate the reductase gene in the sea urchin. In this context it should be noted that the 5.5-kb mRNA encoded by the sea urchin HMG-CoA reductase gene is larger than the 4.7-kb mRNA of hamster (13). It remains to be established whether this increased size is due to a larger coding region or to larger 5' and/or 3'-untranslated regions.

The observed increase (15-fold) in hybridizable sea urchin embryo HMG-CoA reductase mRNA over the course of embryonic development suggests that de novo transcription may contribute to the observed increase in enzyme activity during development. Precedent for such a mode of regulation is found in the case of rat, where diet rich in cholesteryramine and mevinolin result in a 20-fold increase in reductase transcription (25). However, it is clear that in the sea urchin embryo, factors other than transcription regulation may exist because HMG-CoA reductase enzyme activity increased 200-fold between egg and gastrula stage, whereas hybridizable mRNA levels increased only 15-fold over the same time span. Extensive studies in the mouse and rat systems have revealed several potential modes of regulation of HMG-CoA reductase. The enzyme has been shown to be regulated by increased mRNA levels in compactin-resistant cell lines (13), by enzyme activation involving phosphorylation/dephosphorylation cycles (26), by changes in the rates of biosynthesis and/or degradation (27–29), and by changes in activity or degradation as a result of changes in the lipid environment (30, 31). Clearly, some of these mechanisms may play a role during early development of the sea urchin embryo, a time during which extensive cell proliferation and membrane modification occurs.

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Supplementary Material

1. Methods: A protocol for the preparation of the manuscript.
2. References: A list of all references cited in the text.
3. Tables: Additional data or results that are not included in the main text.
4. Figures: Graphs, charts, and images that illustrate the data or results.
5. Appendices: Additional information that is not directly related to the main text but is included for completeness.

EXPERIMENTAL PROCEDURES

Cloning of Embryos — See online protocol for preparation or cloning the zygote of the embryonic sea urchin. The process involved the use of mRNA obtained from fertilized eggs. The mRNA was isolated using the procedure described by Liscum et al. (1984) J. Biol. Chem. 259, 6119-6127.

Isolation of Fragments — Embryos were isolated from the blastula stage and used to prepare the enzyme by homogenization. The enzyme was then purified by gel filtration on a Sephadex column. The purified enzyme was used to prepare the enzyme by gel filtration.

RESULTS

1. HMG-CoA Reductase Activity from Crude Gastrula Supernatant — Activity was expressed as a ratio of HMG-CoA reductase activity to total protein. The activity was measured using a spectrophotometric assay with the enzyme isolated from the gastrula supernatant.

2. HMG-CoA Reductase Activity from Embryonic Sea Urchin Microsomes — Activity was expressed as a ratio of HMG-CoA reductase activity to total protein. The activity was measured using a spectrophotometric assay with the enzyme isolated from the embryonic sea urchin microsomes.
result suggest that the low level of activity in the egg is not due to the presence of an inhibitor that is inactivated over the course of development. However, as shown in Table 1, addition of exogenous egg lysozyme homologues did not inactivate the enzyme in the egg homogenate.

FIG. 1. HMG-CoA reductase activity during sea urchin embryo development. The values expressed are the average of duplicate experiments. See Experimental Procedure for isolation of sea urchin embryos. [Reproduced from Cell (1986).]

Table I

1. Effect of postincubation inhibitors or activators on HMG-CoA reductase activity

| Enzyme Source | % of Total Activity (mg/ml) | Activity Remaining (mg/mg protein) |
|---------------|-----------------------------|----------------------------------|
| Egg microsomes | 105 ± 22 | 195 ± 11 |
| Egg and egg microsomes | 109 ± 15 | 180 ± 11 |
| Egg total homogenate | 83.2 ± 0.5 | 153 ± 13 |
| Egg and egg total homogenate | 103 ± 15 |

The values reported represent the average of at least two different cultures of sea urchin embryos used in duplicate.

2. HMG-CoA Reductase Activity

| Time (hr) | Activity Remaining (mg/mg protein) |
|-----------|-----------------------------------|
| 0         | 105 ± 22                            |
| 1         | 109 ± 15                            |
| 2         | 195 ± 11                            |
| 4         | 83.2 ± 0.5                           |
| 6         | 153 ± 13                            |

The values reported represent the average of two different cultures of sea urchin embryos used in duplicate.

The results suggest that the low level of activity in the egg is not due to the presence of an inhibitor that is inactivated over the course of development. However, as shown in Table 1, addition of exogenous egg lysozyme homologues did not inactivate the enzyme in the egg homogenate.

FIG. 1. HMG-CoA reductase activity during sea urchin embryo development. The values expressed are the average of duplicate experiments. See Experimental Procedure for isolation of sea urchin embryos. [Reproduced from Cell (1986).]

Table II

1. Properties of microsomal HMG-CoA reductase

| Solubilising Agent | % of Total Activity | Activity Remaining |
|--------------------|---------------------|-------------------|
| 5% Glycerol in Buffer A | 30 ± 5               |
| 10% Glycerol in Buffer B | 20 ± 5               |
| 20% Glycerol in Buffer C | 10 ± 5               |
| 30% Glycerol in Buffer D | 5 ± 5                |
| Tris 0.05M, 0.5% | 30 ± 5               |
| 10% Detergent, 1% | 20 ± 5               |
| 10% Glycerol, 1% | 10 ± 5               |
| Salt solution, 1% | 5 ± 5                |
| Sodium deoxycholate, 0.5% | 10 ± 5               |
| Sodium deoxycholate, 0.75% | 5 ± 5                |
| Sodium deoxycholate, 1% | 5 ± 5                |

2. Effect of mild tryptic digestion

| Time (hr) | Activity Remaining (mg/mg protein) |
|-----------|-----------------------------------|
| 0         | 105 ± 22                            |
| 1         | 109 ± 15                            |
| 2         | 195 ± 11                            |
| 4         | 83.2 ± 0.5                           |
| 6         | 153 ± 13                            |

The values reported represent the average of two different cultures of sea urchin embryos used in duplicate.

The results suggest that the low level of activity in the egg is not due to the presence of an inhibitor that is inactivated over the course of development. However, as shown in Table 1, addition of exogenous egg lysozyme homologues did not inactivate the enzyme in the egg homogenate.

FIG. 1. HMG-CoA reductase activity during sea urchin embryo development. The values expressed are the average of duplicate experiments. See Experimental Procedure for isolation of sea urchin embryos. [Reproduced from Cell (1986).]
Embryonic Sea Urchin HMG-CoA Reductase

Analysis of Sea Urchin HMG-CoA Reductase mRNA During Development — To provide further evidence that the 6.43 kb Pol-IIIA fragment encodes a form of the sea urchin HMG-CoA reductase, we assessed its ability to cross-hybridize with the 4.3 kb herring mRNA described by Matthes et al. (1983). As shown in Fig. 1, lane 2, we detected the 4.3 kb herring HMG-CoA reductase mRNA using the homologous 6.43 kb Pol-IIIA fragment derived from pCH63. The results in Fig. 1, lane 2, indicated that the sea urchin 6.43 kb Pol-IIIA fragment of pCH63 cross-hybridized to this same 4.3 kb RNA; this observation is consistent with the possibility that this gene is the same gene described by Matthes et al. (1983).

To determine the level of sea urchin HMG-CoA reductase mRNA, the 4.43 kb Pol-IIIA fragment was hybridized to total RNA, poly A RNA and poly (A)$^+$ RNA isolated from the different stages of sea urchin development, as described in Materials and Methods. The levels of the sea urchin embryo reductase mRNA were normalized to an arbitrary olfactory mRNA which remains constant during development. The level of this mRNA increased 10-fold from the egg to the gastrula stage, after which it decreased approximately 1.5-fold at the polychaete stage. By the 18-cell stage, the level of the mRNA had increased 2-fold at the mesenchyme blastula stage. The level of mRNA has increased 10-fold over the level fixed at unfertilized eggs. The herring 4.3 kb Pol-IIIA fragment of pCH63 also hybridized to the same developmentally expressed mRNA data not shown. It seemed possible that the section of the transcript containing the 6.43 kb Pol-IIIA fragment might be the reason for differences between the increasing portion of this genomic fragment and these RNA species. To explore this possibility, we hybridized the 6.43 kb fragment containing coding sequence and a 4.3 kb fragment containing no coding sequence. The important coding and noncoding sequences were hybridized to sea urchin embryo RNA. As shown in Figure 7, left panel, the fragment containing coding sequence was only present at a 6.5 kb mRNA at all stages of development, and this mRNA increases 15-fold as described similarly in Figure 6. Conversely, the noncoding fragment (Figure 7, right panel) only hybridizes to the minor mRNA species at all stages of development.

FIG. 5 - Cross-hybridization of C-148 RNA with the 6.43 kb Pol-IIIA sea urchin genomic fragment and the 4.3 bp Pol-IIIA sea urchin cDNA fragment. C-148 total RNA (10 $\mu$g) was electrophoresed and transferred to nitrocellulose. The RNA was hybridized to the herring HMG-CoA reductase probe, C-148 cDNA probe, and the 1.8 kb T3-RU. The blot was washed in SSPE, 0.1% SDS, 60°C, 1 hr. The autoradiograph is shown in Fig. 6. The experiment was repeated with E. coli RNA as a negative control.

FIG. 7 - Analysis of sea urchin HMG-CoA reductase mRNAs levels during development. Equivalent amounts (10 ug) of total RNA, poly A RNA and poly (A)$^+$ RNA were electrophoresed and transferred to nitrocellulose as described in Materials and Methods. The RNA was hybridized to the 6.43 kb Pol-IIIA genomic fragment, and the filter was exposed to Kodak X-OMAT AR-5 film at 68°C for 24 h. #1: Total RNA. #2: poly A RNA. #3: poly (A)$^+$ RNA. 16-cell embryos were harvested at 95 h presumptive hatching at 65 h, and polychaete stage embryos at 93 h post-fertilization.