GLYCOPROTEIN SYNTHESIS AND EMBRYONIC DEVELOPMENT

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I. INTRODUCTION

In recent years considerable progress has been made in understanding the basic mechanism of synthesis of N-glycosidically linked oligosaccharide chains of glycoproteins.[1] In contrast, the processes that regulate the biosynthesis of these glycoproteins and their biological function still are not well understood. As shown in Figure 1, there are two general classes of N-glycosidically linked oligosaccharide chains, one containing a polymannose chain and a second composed of a complex chain that terminates in sialic acid units. It is apparent from an examination of the structure of these two classes of oligosaccharide chains that they have a common core consisting of two N-acetylglucosamine residues and three mannose residues. However, it now is apparent that they share more than this common core. Indeed, it appears that the mature oligosaccharide chains in both classes of N-linked glycoproteins arise from a common, larger oligosaccharide precursor. As shown in Figure 2, the biosynthesis of this common oligosaccharide precursor occurs through a rather novel series of reactions, involving sequential attachment of the sugars to a molecule of the phosphorylated polyprenol, dolichylphosphate. In this process the added sugars either are derived directly from their sugar nucleotides or from preformed glycosylphosphoryldolichol derivatives. The end result is assembly of a large oligosaccharide still attached to dolichol via a pyrophosphate bridge.

Subsequently, the oligosaccharide, as shown in Figure 3, is transferred to an appropriate Asn residue on the growing polypeptide chain in a cotranslational process. After completion of the glycoprotein it is subjected to processing reactions that lead to the formation of either complex or polymannose-type oligosaccharide chains. These reactions, which have been studied extensively in higher eukaryotic organisms, are known to occur within the internal membrane system consisting of the endoplasmic reticulum and the Golgi complex.

II. EMBRYONIC DEVELOPMENT IN SEA URCHINS

In view of the radically changing cell-cell interactions during embryogenesis and the possibility that glycoproteins are involved in such interactions, we decided some years ago to undertake a study of the synthesis and possible functions of these molecules in sea urchin embryos. Sea urchins were chosen because a large number of embryos can be cultivated synchronously in a simple culture medium consisting of seawater. Furthermore, the sea urchin system has been studied extensively by biologists for over a hundred years and, consequently, there is a wealth of information available on the morphogenetic changes involved in embryonic development in these organisms.[2] Some of the key events

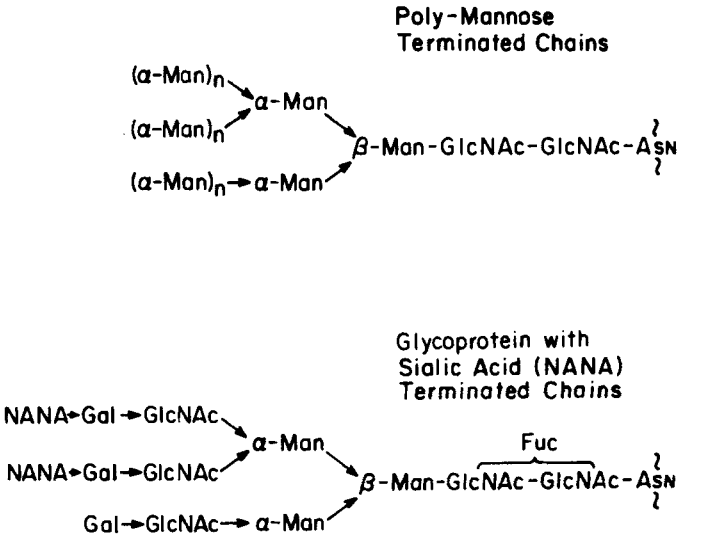

FIGURE 1. Structure of the typical polymannose and complex-type N-linked oligosaccharide chains found in glycoproteins.

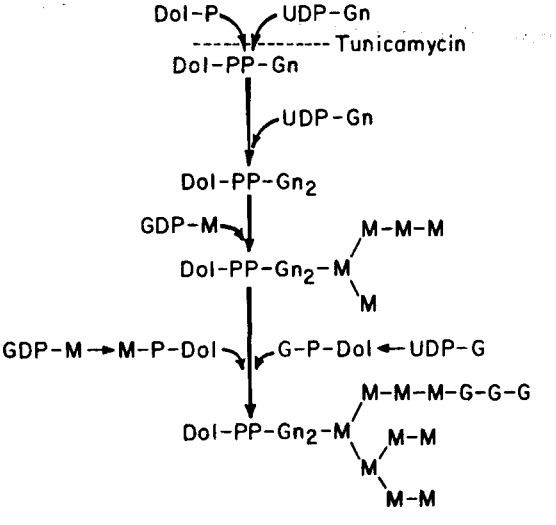

FIGURE 2. Key steps involved in the synthesis of the oligosaccharide chain attached to dolichylpyrophosphate. The site of inhibition of synthesis of the oligosaccharide-lipid by tunicamycin is shown. Dol-P — dolichyl phosphate; Gn — N-acetylglucosamine; M — mannose; G — glucose.

in the developmental changes are diagrammed in Figure 4. Shortly after fertilization of the egg, the resulting zygote undergoes its first cleavage. Subsequent development of the two-stage embryo involves additional cleavages. At the 16-cell stage, the first unequal cleavage occurs, and three cell types known as the micro-, meso-, and macromeres are discernable. Continued cell division leads to a solid ball of cells known as the morula. Further enlargement and hollowing of this ball of cells results in formation of the blastula stage embryo. Upon completion of blastulation, the embryo hatches from the rigid envelope in which it has developed up to this stage. The hatched blastula now contains

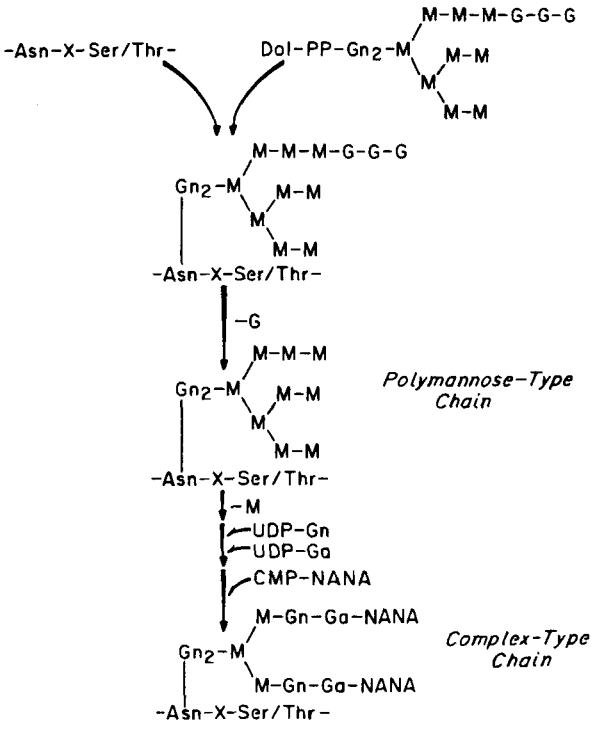

FIGURE 3. Transfer of the oligosaccharide chain from dolichylpyrophosphate to a protein acceptor, and subsequent processing to yield either polymannose or complex type chains. Ga — galactose; NANA — N-acetyl neuramuric acid.

within the hollow cavity known as the blastocoel the primary mesenchyme cells. These cells ultimately line up, fuse, and synthesize a primitive skeleton (spicule) composed of $CaCO_3$.

At the next stage of development, gastrulation, a striking morphorgenetic event that occurs in all developing higher organisms, is initiated. This process involves invagination of the ectoderm layer, leading ultimately to formation of a primitive gut. As the invagination is initiated an additional class of cells, known as secondary mesenchyme cells, is apparent at the leading portion of the developing gut. These cells, which will be discussed in more detail later, send out long pseudopodia that appear to interact with the ectodermal layer where eventually fusion and formation of a complete gut tube will occur. Upon completion of the gut tube and further elongation of the spicules, the embryo has developed to the prism stage. Further morphogenetic events lead to the formation of the pluteus stage embryo. At this stage, for the first time, the embryo begins to feed and further development is arrested in the absence of nutrients.

Prior to this stage, the embryo either has stores or has the ability to make all the organic molecules that are involved in this complex series of morphogenetic changes. Earlier studies have established that immediately following fertilization polypeptide synthesis is initiated utilizing stores of mRNA that were previously made during oogenesis. This is followed later by a turn-on in the synthesis of RNA and DNA.[3–5] At the gastrulation stage there first appears a requirement for synthesis of new classes of molecules. Thus, for example, embryos can be cultivated up to the hatched blastula stage in the absence of inorganic SO_4. However, in the absence of this ion, gastrulation does not ensue, probably because sulfated proteoglycans cannot be synthesized.[6,7] Further-

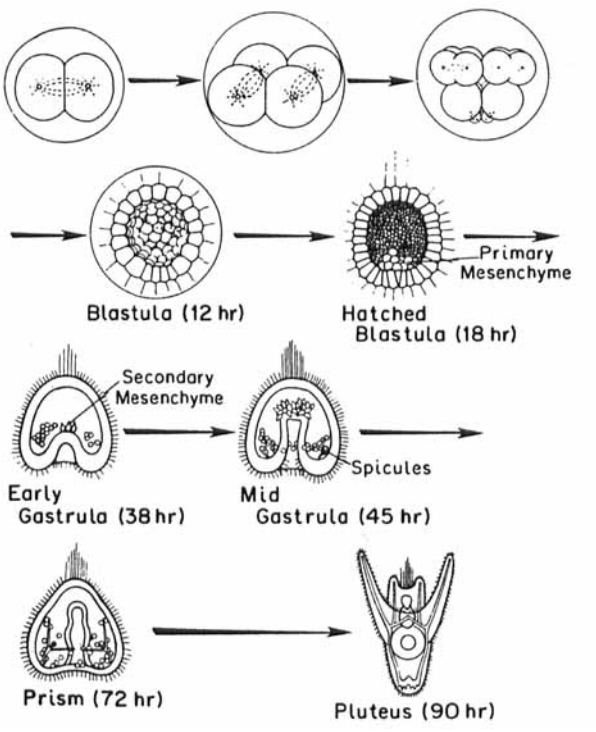

FIGURE 4. Some of the key steps in the development of a *S. purpuratus* embryo to the pluteus stage.

more, experiments using the RNA inhibitor, actinomycin, indicate that the first developmental stage at which new mRNA, and presumably new translation products, must be made is gastrulation.[5]

III. EFFECT OF INHIBITION OF GLYCOSYLATION ON GASTRULATION

We initiated our studies on glycoprotein synthesis in developing sea urchin embryos by studying the East Coast sea urchin, *Arbacia punctulata,* and later directed our continued efforts to the West Coast urchin, *Stronglyocentrotus purpuratus.* In the first experiments we studied the possible effect of inhibition of synthesis of the oligosaccharide chains of N-linked glycoproteins by the drug tunicamycin on embryonic development.[8,9] As indicated in Figure 2, tunicamycin is known to block the very first step in the assembly of the oligosaccharide chain; i.e., transfer of N-acetylglucosamine-l phosphate from UDP-N-acetylglucosamine to dolichylphosphate. Much to our surprise, when we cultivated very early embryos in the continued presence of tunicamycin, there was no effect on any of the early cell cleavages, as well as on formation of the morula, blastula, and hatched blastula stage embryo. However, as shown in Figure 5, at the hatched blastula stage development is arrested and gastrulation does not occur. Thus, it appeared that gastrulation was the first developmental stage that was sensitive to tunicamycin. Control studies revealed that this effect of tunicamycin was not the result of toxicity, since the embryos respired normally and carried out normal rates of DNA and RNA synthesis. In order to better define the effect of tunicamycin on glycoprotein synthesis, we studied the synthesis and glycosylation of proteins by utilizing labeled amino acids and glucosamine as precursors.[9] As shown in Figure 6, the rate of amino acid incorporation in protein

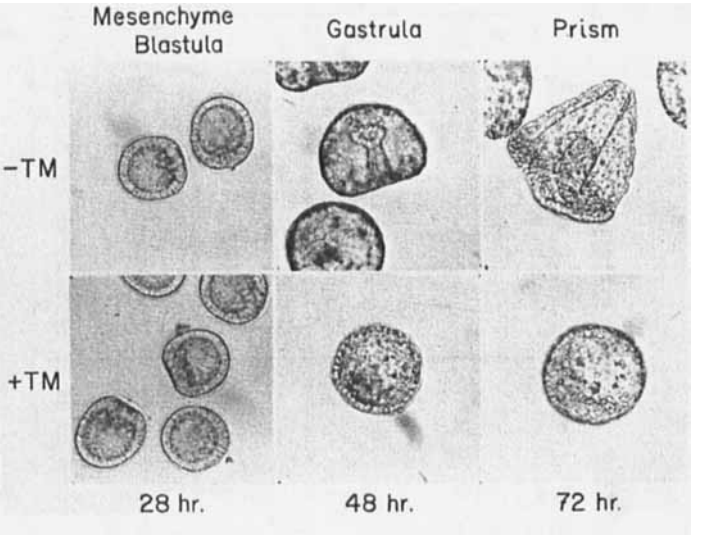

FIGURE 5. Photomicrographs showing the effect of tunicamycin on the development of embryos. At the mesenchyme blastula stage, just prior to gastrulation, no effects of tunicamycin are yet apparent. However, the subsequent gastrulation process is totally blocked in the presence of tunicamycin.

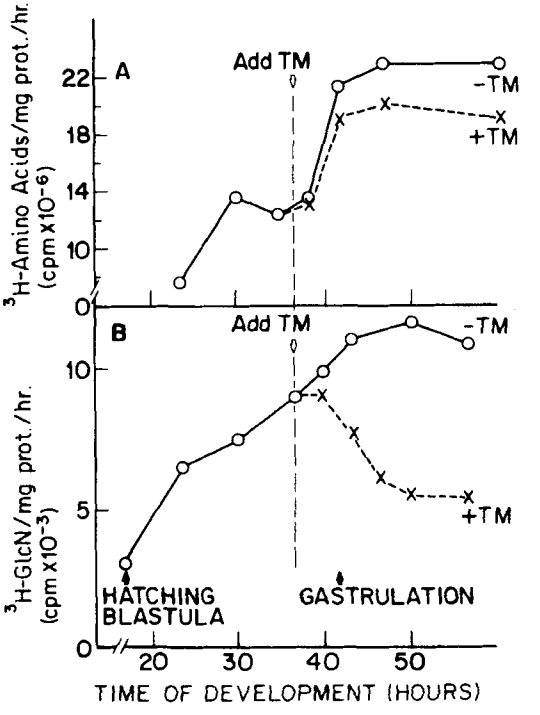

FIGURE 6. Tunicamycin has little effect on the rate of amino acid incorporation into proteins, but causes a marked inhibition in the rate of glycosylation of proteins.

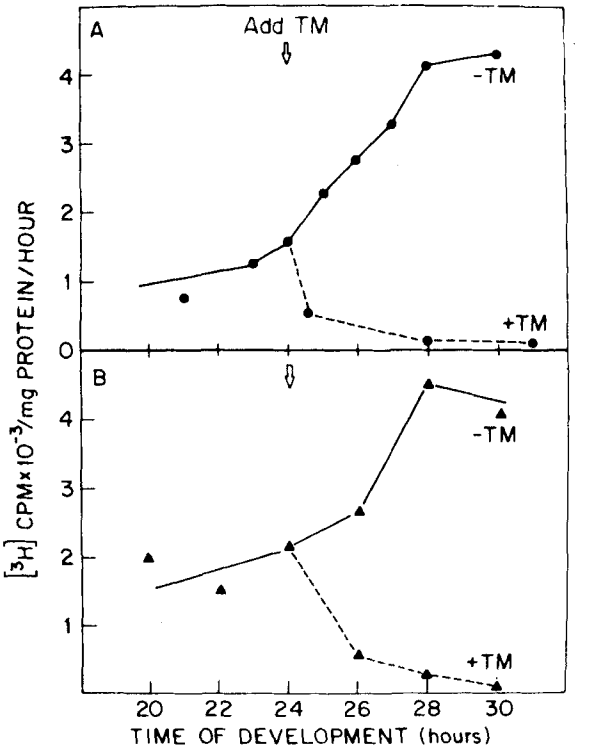

FIGURE 7. Addition of tunicamycin to embryos results in a marked decrease in the rate of oligosaccharide-lipid synthesis. It is apparent that as the control embryos develop into the gastrulation stage, the rate of oligosaccharide pyrophosphoryl dolichol synthesis markedly increases. In (A) oligosaccharide-lipid synthesis was measured using labeled glucosamine as precursor, while in (B) labeled mannose was used. At the arrow tunicamycin (TM) was added to an aliquot of the culture.

increases over development, and the addition of tunicamycin has relatively little effect on this process. When glycosylation is studied, a similar increase in the rate is observed, but in this case there is a profound inhibitory effect of tunicamycin. This finding, indicating that the effect of tunicamycin was rather specific, was further documental by studying the effect of tunicamycin on assembly of the oligosaccharide-lipid that serves as a precursor of these glycoproteins. When either mannose (Figure 7A) or glucosamine incorporation (Figure 7B) into oligosaccharide lipid was measured two facts were apparent. First, there is a marked increase in the rate of assembly of oligosaccharide-lipid over the period of development studied. Second, synthesis of this molecule is rapidly and almost completely inhibited by addition of tunicamycin. Recently we have correlated this enhanced rate of oligosaccharide synthesis near the time of gastrulation with the overall process of N-linked glycoprotein synthesis.[10] In Figure 8, the rate of synthesis of all of the polymannose-type glycoproteins is shown over the course of embryonic development. It is apparent that there is little, if any, synthesis of N-linked glycoproteins prior to the hatched blastula stage. Only just prior to the onset of gastrulation is there significant N-linked glycoprotein synthesis, and the rate of this process accelerates throughout gastrulation and prism formation. This finding is, of course, consistent with the earlier observation that tunicamycin had no effect on stages of development earlier than gastrula. Thus, it appears that the enbryos can go through all of the many cleavages and

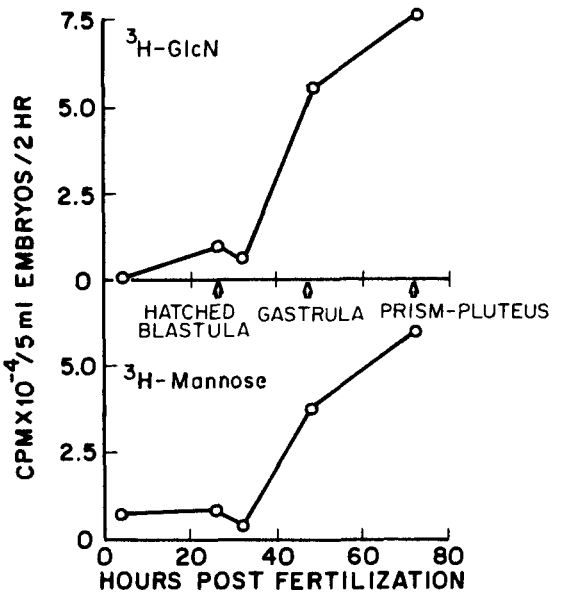

FIGURE 8. Developmental profile of synthesis of glycoproteins containing polymannose chains. It is clear that prior to gastrulation stage there is little or no synthesis of these proteins, but that their rate of synthesis rapidly increases at time of gastrulation.

earlier developmental events without any *de novo* synthesis of N-linked glycoproteins. Only at the gastrulation stage are newly synthesized molecules of this type required.

IV. EFFECT OF INHIBITION OF DOLICHOL SYNTHESIS ON GASTRULATION

Because the embryo at these early stages of development is a closed system requiring only oxygen and inorganic salts in the culture medium, it seemed possible that during or prior to gastrulation developmental changes in dolichol synthesis might occur. Therefore, using labeled acetate as a precursor, the rate of synthesis of dolichol and cholesterol in developing embryos was studied. As shown in Figure 9, immediately after fertilization there is a rapid increase in the rate of synthesis both dolichol and dolichylphosphate which reaches its maximum midway between the hatched blastula and gastrula stage. Cholesterol synthesis, which occurs at a level at least tenfold greater than that of dolichol, also is turned on immediately after fertilization, and reaches its maximum after gastrulation. With this information in hand, we next investigated the effect of compactin on both embryonic development and on dolichol, dolichylphosphate, and sterol biosynthesis.[11,12] As shown in Figure 10, compactin blocks the activity of HMG-CoA reductase, resulting in inhibition of mevalonic acid biosynthesis. Mevalonic acid is known to be a common precursor to not only cholesterol and coenzyme Q, but to dolichol. In initial experiments we found that the presence of compactin in the culture medium had no effect on any of the early stages of development, and it was only just prior to gastrulation that the embryos arrested. Thus, compactin, like tunicamycin, appears to specifically affect the gastrulation process. As shown in Table 1, under conditions where compactin inhibited normal gastrulation by 75%, the incorporation of acetate into cholesterol virtually was abolished completely and inhibition of dolichol synthesis was

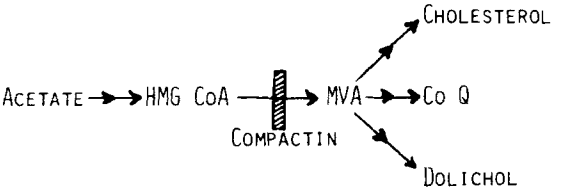

FIGURE 9. Rates of synthesis of dolichylphosphate, dolichol, and cholesterol during embryonic development. Addition of compactin beyond the cross-hatched time does not result in a block to gastrulation.

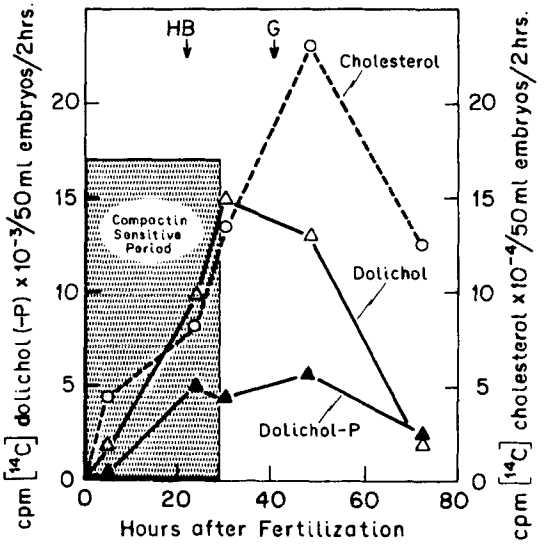

FIGURE 10. Pathway for the synthesis of cholesterol, coenzyme Q, and dolichol.

Table 1
COMPACTIN INHIBITS BOTH STEROL AND DOLICHOL SYNTHESIS

Addition	Normal gastrula (%)	CPM ^{14}C-acetate incorporated into Sterol	Dolichol
None	96	33,200 (100%)	3,073 (100%)
Compactin	25	456 (1.4%)	548 (18%)

Note: Compactin (20 μM) was added 4 hr after fertilization. At the time of gastrulation (48 hr) the embryos were labeled for 2 hr with ^{14}C-acetate.

approximately 80%. When the effect of addition of compactin at various stages of development was studied, it was found, as shown in Figure 9, that after the hatched blastula stage the embryos were no longer sensitive to compactin. Given the fact that dolichol and dolichylphosphate synthesis reach maximal rates shortly after the hatched

Table 2
EFFECT OF SUPPLEMENTATION WITH ISOPRENOID-DERIVED LIPIDS ON DEVELOPMENT OF EMBRYOS CULTURED IN PRESENCE OF COMPACTIN

Supplement[a]	Compactin (20 μM)	Normal gastrula observed[b] (%)
None	−	89—98
None	+	4—18
Cholesterol, coenzyme Q_{10}, dolichol	+	75—89
Cholesterol, coenzyme Q_{10}	+	2—12
Dolichol	+	61—70
Cholesterol, dolichol	+	73
Coenzyme Q_{10}, dolichol	+	75
Dolichylphosphate	+	84

[a] Supplements were added as ethanolic solutions. The final concentrations of cholesterol, coenzyme Q_{10}, dolichol, and dolichylphosphate used were 20, 0.1, 1, and 5 $\mu g/m\ell$, respectively. These concentrations of lipids had no effect on the development of embryos that had not been treated with compactin.

[b] Under each of the culture conditions, at least 200 embryos were examined microscopically at 48 hr after fertilization. Ranges of values shown are those obtained in three separate experiments.

blastula stage, it appears that the embryo must synthesize some minimal level of dolichol and/or dolichylphosphate in order to gastrulate. Perhaps once this has been accomplished, no further synthesis of dolichol is necessary for gastrulation.

At this stage in our studies, however, such an idea was purely speculative, since it was apparent that the compactin inhibited not only dolichol but cholesterol synthesis as well. Therefore, we undertook a simple nutritional experiment in order to determine if the block of the polyisoprenoid pathway and the concomitant arrest of development were induced specifically due to a deficiency in dolichol. As shown in Table 2, embryos were cultivated in the absence and presence of compactin. In addition, a third batch of embryos was cultivated in the presence of compactin but also was supplemented by addition of cholesterol, dolichol, and coenzyme Q to the culture medium. It is apparent that supplementation with all three compounds results in restoration of gastrulation to near control levels. It also is clear that of these three compounds, the only one active in obviating the compactin-induced developmental block is dolichol or its derivative, dolichylphosphate. Thus, on the basis of this experiment, it may be concluded that the embryos do, indeed, have a specific requirement for ongoing dolichol synthesis during the stages of development preceding gastrulation.

To determine if the inhibition in dolichol synthesis caused by compactin treatment and the ability of exogenous dolichol to obviate the block in gastrulation by compactin had an effect on glycosylation processes, mannose labeling experiments were carried out and the synthesis of mannosylphosphoryldolichol, the oligosaccharide-lipid, and N-linked glycoproteins was measured (Table 3). It is apparent that compactin had a profound inhibitory effect on synthesis of the dolichol-linked saccharides and on protein glycosylation. However, in embryos cultivated in the presence of both compactin and

Table 3
EFFECTS OF COMPACTIN AND DOLICHOL SUPPLEMENTATION ON SYNTHESIS OF MANNOSE-LABELED GLYCOPROTEINS AND LIPIDS

Supplement	Incorporation of [^{3}H]mannose into[a]		
	Mannosyl-lipid	Oligosaccharide-lipid	N-linked glycoproteins
None	100	100	100
Compactin	41—46	9—11	56
Compactin and dolichol	96—129	168—230	177

[a] Values are expressed as percent of control values. Ranges of values obtained from duplicate experiments are provided for saccharide-lipids. All analyses were performed on 24- to 26-hr embryos.
[b] N-linked glycoprotein synthesis was assessed by isolation of N-linked glycopeptides.

dolichol, this inhibition was abolished. Under these conditions glycosylation of proteins, as well as synthesis of the lipid intermediates, was enhanced over control levels. This appears not to be merely a quirk of this embryonic system, since in experiments in other systems similar enhancement of protein glycosylation by addition of dolichylphosphate to the culture medium has been observed.[13] Thus, as suggested by others, it appears that at least in some situations dolichylphosphate may be rate limiting in the glycosylation of N-linked proteins.

Experiments using radioactive acetate as a precursor can be misleading because the internal pool of unlabeled acetate may change during development. Because this parameter is difficult to measure, we developed methods to chemically quantitate dolichol, dolichylphosphate, and cholesterol, and determined the levels of these three compounds in embryos as a function of development. As shown in Figure 11, no dolichylphosphate is present in eggs, but there is an increase in the level of dolichylphosphate during development, with the maximum level being found at gastrula stage. In contrast, dolichol is present in the egg and its level remains relatively constant until gastrulation. At this point the level of dolichol increases while that of dolichylphosphate decreases. It is of interest to compare these findings with those obtained by measuring *de novo* synthesis of dolichol and dolichylphosphate with labeled acetate (Figure 10). It is apparent that the two types of experiments give rather different results, since in the case of *de novo* synthesis the predominant product appears to be dolichol, not dolichylphosphate. The reason for this apparent discrepancy is not clear, but it should be noted that the late stages in the pathway of biosynthesis of dolichylphosphate are not known. As shown in Figure 12, assembly of the dolichol backbone involves sequential addition of isopentenyl pyrophosphate units to the growing polyisoprenoid chain. However, the end product of the pathway per se is not known. As shown, one of the later steps must be a reduction to produce either dolichol or dolichylphosphate. Whether or not this reduction is preceded or followed by removal of the pyrophosphoryl group, or by removal of one of the phosphates from the pyrophosphoryl group, is unknown. In relation to this question it is important to note that the kinase and phosphatase depicted in Figure 12 have been reported in a variety of biological systems.[14-22] Obviously, these two enzymes may play a role in regulating the levels of dolichol and dolichylphosphate.

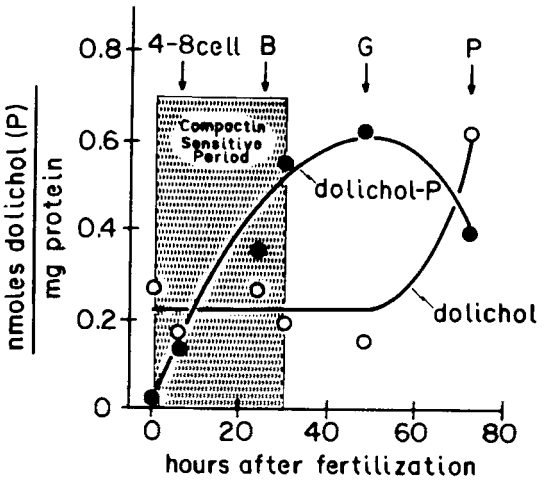

FIGURE 11. Dolichol and dolichylphosphate content of embryos during development.

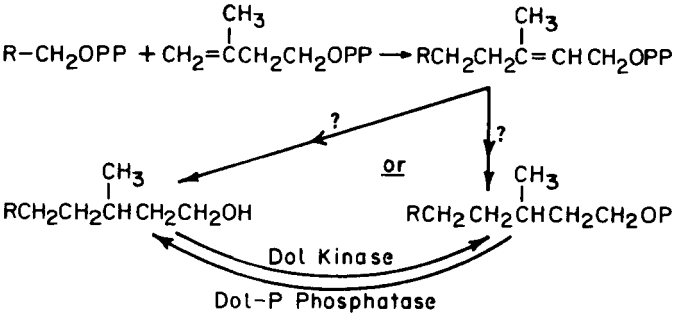

FIGURE 12. Possible reactions involved in the synthesis and/or the interconversion of dolichol and dolichylphosphate. Following addition of the final isopentylpyrophosphate unit to the growing polyprenol chain two possible routes for formation of dolichyl phosphate are shown. In one case dolichol is formed by reduction and hydrolysis of the pyrophosphate group. Subsequent phosphorylation of dolichol via a CTP-dependent kinase would produce dolichyl phosphate. In the other case reduction and hydrolysis of one phosphate residue results in formation of dolichyl phosphate. Conversion of dolichyl phosphate to dolichol could be mediated by the action of dolichyl phosphate phosphatase.

V. DEVELOPMENTAL REGULATION OF DOLICHOL PHOSPHORYLATION

In the hope of gaining further insight into regulation of the level of dolichylphosphate in the sea urchin embryo, we studied the synthesis of dolichylphosphate using $^{32}P_i$ as a precursor. As shown in Figure 13, when the rate of incorporation of $^{32}P_i$ into dolichol was measured over the course of development, it was found that maximal synthesis of dolichylphosphate occurs midway between hatching blastula and gastrula. However, as illustrated in Figure 12, formation of $^{32}P_i$-labeled dolichylphosphate could occur in two ways: by kinase-mediated phosphorylation of endogenous dolichol or by *de novo* synthesis from $^{32}P_i$-labeled isopentenylpyrophosphate. For this reason we turned from

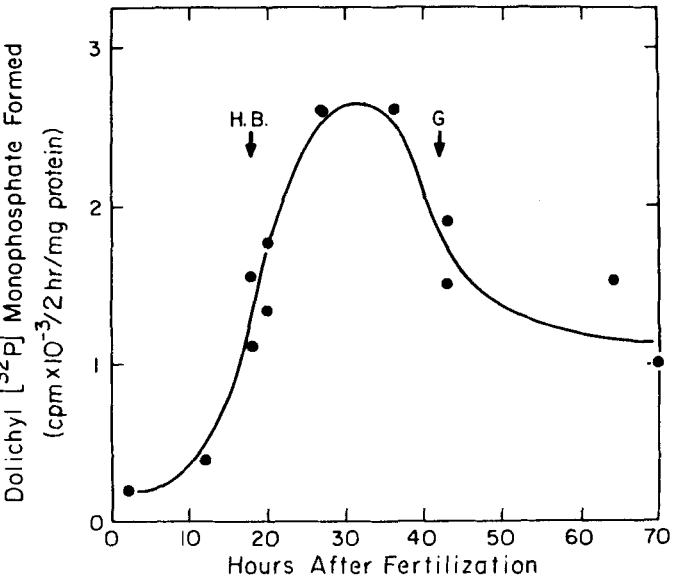

FIGURE 13. Synthesis of labeled dolichylphosphate from $^{32}P_i$ during embryonic development.

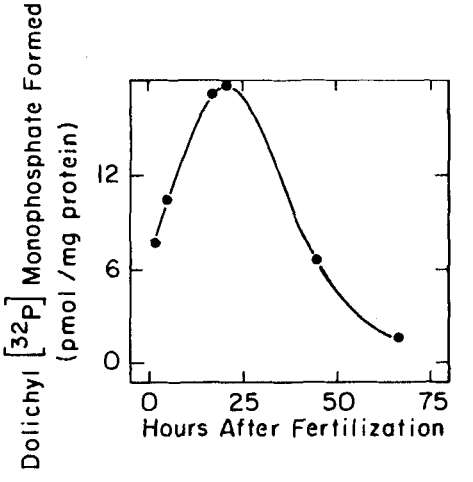

FIGURE 14. Dolichol kinase activity during embryonic development

the in vivo system and investigated the possibility that dolichol kinase was present in membrane preparations prepared from the embryos at various stages of development. In collaboration with Dr. C. J. Waechter it was found that, indeed, such membranes contained dolichol kinase activity. Moreover, developmental studies revealed that there is a striking increase and subsequent decrease in the activity of dolichol kinase over the course of development. As shown in Figure 14, the maximal activity of dolichol kinase in membrane preparations is observed relatively early in development, at the hatched blastula stage; at all later stages there is a progressive decrease in activity. Very recently, in collaboration with Drs. M. Scher and C. J. Waechter, we have examined the levels of one of the other enzymes that may be involved in regulating the level of

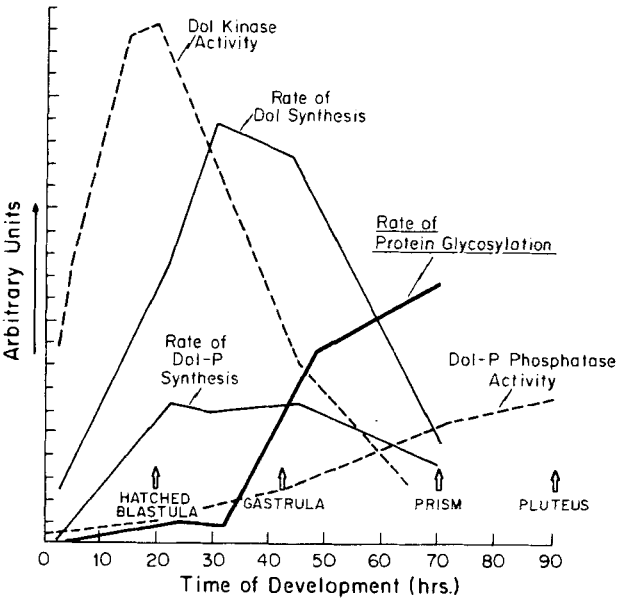

FIGURE 15. Summary of developmental changes associated with glycoprotein synthesis during embryonic development.

dolichylphosphate, dolichylphosphate phosphatase.[23] It has been found that this enzyme activity shows a developmental profile that is strikingly different from that of dolichol kinase. Phosphatase activity is very low early in development, but continually increases to a high level as the embryos pass through the gastrula stage to the pluteus stage. Thus, early in development, during which time there is rapid increase in the rate of *de novo* synthesis of dolichol and dolichylphosphate, dolichol kinase increases to a maximum. Subsequently, the activity of this enzyme decreases while the activity of dolichylphosphate phosphatase rapidly increases.

These dolichol-related changes, as well as changes in glycoprotein synthesis over the course of development, are summarized in Figure 15. It is apparent that the earliest change yet detected in processes related to glycoprotein synthesis is an increase in the activity of dolichol kinase. After this activity reaches a maximum and starts to decline, the synthesis of dolichol and dolichylphosphate are maximal. Midway between hatched blastula and gastrula, N-linked glycoprotein synthesis is first observed, and the rate of this process accelerates during gastrulation. Finally, as indicated earlier, dolichylphosphate phosphatase activity is low at a time where the kinase is high, and continually increases over the course of development. Although these recent studies on the kinase and the phosphatase have not yet clarified the question of the end product of the pathway for *de novo* biosynthesis of dolichol, it seems likely that both enzymes play an important role in regulating dolichol metabolism. Further studies hopefully will shed more light on the question of the terminal steps in dolichol synthesis.

VI. REGULATION OF THE TRANSLATION OF GLYCOSYLATABLE PROTEINS

Another important question relating to induction of glycosylation during development concerns the polypeptide acceptors that are glycosylated at gastrulation. It is known from a number of studies that N-linked glycosylation is a cotranslational process that occurs in the rough endoplasmic reticulum. Furthermore, it is clear that the Asn residues

that become glycosylated must be part of the sequence -Asn-X-Ser/Thr-.[1] As noted earlier, translation of polypeptides is turned on shortly after fertilization. However, it was unclear if glycosylatable proteins, i.e., those containing -Asn-X-Ser/Thr- sites, are synthesized throughout early stages of development, but only those being synthesized at the time of gastrulation become glycosylated. An alternative possibility is that such proteins are not translated prior to gastrulation, and that the initiation of glycoprotein synthesis at gastrulation involves not only induction of the enzymes involved in the glycosylation pathway, but expression of new messages directing the translation of these glycosylatable proteins. We have begun to investigate this question using an in vitro coupled translation-glycosylation system programmed with mRNA isolated from embryos at the various stages of development.[24] Preliminary results indicate that the messages for certain glycosylatable proteins are present throughout early development, whereas messages for others are newly transcribed just before gastrulation. Further work is in progress to document this preliminary conclusion.

VII. POSSIBLE FUNCTION OF N-LINKED GLYCOPROTEINS IN GASTRULATION

The findings that N-linked glycoprotein synthesis is first observed in the embryos at the time of gastrulation, and that a blockage in the synthesis of these molecules either by the use of tunicamycin or compactin, raise the interesting question of the function of the N-linked oligosaccharide chains in the gastrulation process. As shown by Akasaka et al.[25] at the University of Tokyo, the secondary mesenchyme cells play a key role in the gastrulation event. Scanning electron micrographs revealing the cellular organization in the blastocoel, as shown in Figure 16, indicate that the secondary mesenchyme cells at the tip of the advancing gut send out long pseudopodial processes that interact with both the inner face of the ectodermal cells and with the endodermal cells at the top of the developing gut tube. It is not clear, of course, whether these pseudopodial processes play a role in a guidance system directing the advancing primitive gut to the ectoderm or if they are actually involved in facilitating the invagination process. In either case, it seems possible that the N-linked glycoproteins newly synthesized at gastrulation may play a key role in the interactions between the secondary mesenchyme cells and the ectoderm. Indeed, as Akasaka et al[25] have shown, in agreement with our earlier findings, treating embryos with tunicamycin causes an arrest in gastrulation. Electron microscopic examination of such treated embryos revealed disorganization and fragmentation of both the ectodermal cells and the mesenchyme cells.

Our working hypothesis for the function of glycoproteins during gastrulation is that they interact with complementary binding proteins (lectins?) and that this interaction is essential for gastrulation. We plan to test this hypothesis by preparing antibodies to the glycoproteins first made at gastrulation. Microinjection of such antibodies into the blastocoel of early gastrula embryos would be expected to result in a block to gastrulation if the proposed interaction plays a key role in the invagination event. Additionally, the use of tagged antibodies should allow us to determine if synthesis of N-linked glycoproteins is localized to a specific subclass of cells in the ectoderm or endoderm, or to the secondary mesenchyme cells.

VIII. CONCLUSIONS

In summary, the sea urchin embryo has proven to be a useful model to study a number of processes associated with the synthesis of N-linked glycoproteins. In these organisms,

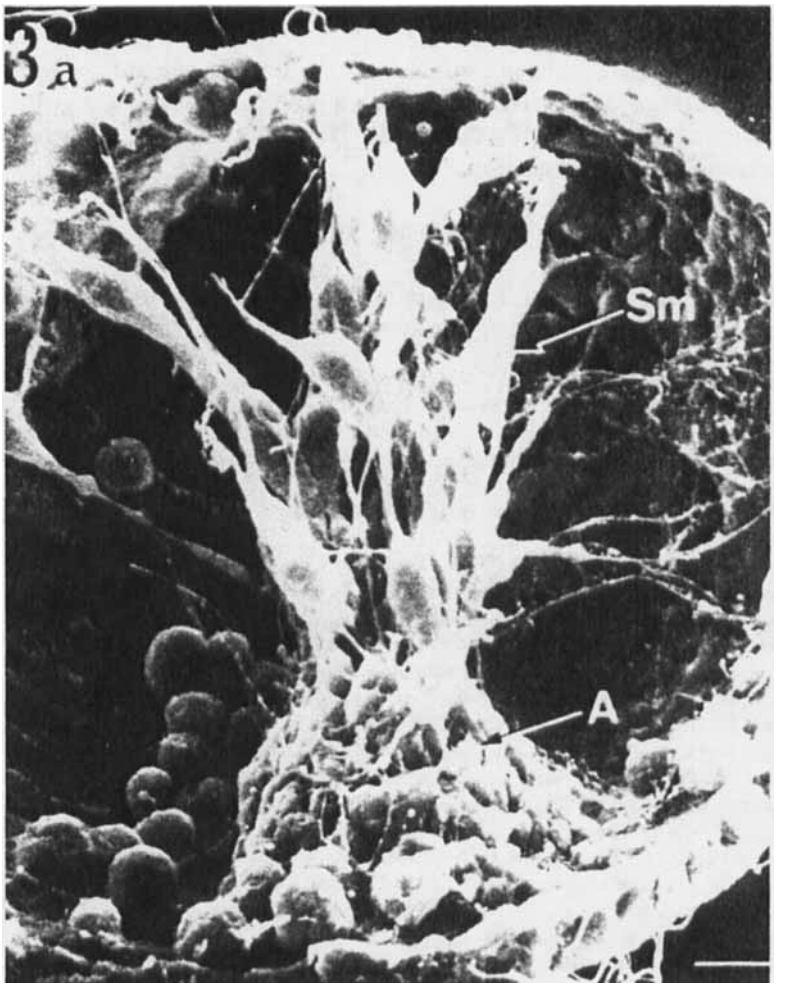

FIGURE 16. Scanning electron micrograph of a sea urchin embryo at early gastrulation. The advancing gut (A) and the secondary mesenchyme (S.m.) are labeled. (Reproduced from Akasaka, K., Amemiya, S., and Terayama, H., *Exp. Cell Res.*, 129, 1, 1980. With permission.)

N-linked glycoproteins are not synthesized prior to gastrulation. At this stage, synthesis of N-linked glycoproteins occurs for the first time, and this process is essential for gastrulation. A series of prior events set the scene for the onset of glycoprotein synthesis. These include early induction of dolichol kinase and synthesis of dolichylphosphate and dolichol. It also seems likely that stored, silent mRNA, as well as newly synthesized mRNA, directing the synthesis of the glycosylatable proteins are first expressed at this time. Later, after glycoprotein synthesis is well under way, the level of dolichylphosphate phosphatase increases markedly and dolichol kinase decreases. It remains to be established, however, whether these processes are also accompanied by changes in the activity of the enzymes involved in the transfer of the oligosaccharide from dolicylpyrophosphate to the newly synthesized proteins. Future studies should provide insight into the even more interesting question of the function of these N-linked glycoproteins in the gastrulation process.

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