Stage-specific protein synthesis during early embryogenesis in *Drosophila melanogaster*

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The changes in protein species synthesized during early *Drosophila* embryogenesis were characterized by two-dimensional electrophoresis. Of the 261 proteins scored, 68 (26%) show dramatic changes in rates of synthesis during the first 8 h of embryogenesis. These stage-specific proteins can be classified into three categories: early, detected at 1, 2 and 3 h but not later; late, not detected at 1 h, but appearing later; and discontinuous, detected before and after, but not at 3 and 4 h. RNA was extracted from three representative stages, translated *in vitro*, and the translation products separated on two-dimensional gels. There was a strong correlation between the patterns of synthesis *in vivo* and *in vitro*, suggesting that the early proteins are translated from maternal mRNA, and the late proteins from zygotic mRNA. A thorough comparison was made between the proteins synthesized in wild-type and *dorsal* embryos, in which virtually only dorsal hypoderm differentiates. The first observed difference was a reduced synthesis of actin I at 8 h, indicating that the absence of mesodermal and endodermal tissues is not detectable at the level of moderately abundant protein until the onset of differentiation.

Key words: *Drosophila*/embryogenesis/*dorsal*/two-dimensional gels/protein synthesis

Introduction

The general pattern of gene expression during early embryogenesis has several common features in the organisms that have been most intensively studied. During the first few cleavage divisions, the level of transcription in embryonic nuclei is very low, but increases dramatically at a specific time (in *Xenopus laevis*, Newport and Kirschner, 1982; in *Drosophila*, Zalokar, 1976; Anderson and Lengyel, 1979). Protein synthesis is initially directed by the large quantity of maternally-derived mRNA, which is gradually replaced by transcripts from the embryonic genome. Analyses of the sequence composition in sea urchin (Davidson, 1976), *X. laevis* (Perlman and Rosbash, 1978), and *Drosophila* (Arthur *et al.*, 1979) have demonstrated that most mRNA sequences are present throughout embryogenesis, and only a small fraction of the moderately abundant sequences appear or disappear. These results have been confirmed by measurements of the concentrations of individual mRNA species by hybridization to cDNA clones in *Xenopus* (Dworkin and Hershey, 1981) and sea urchin (Flytzanis *et al.*, 1982).

Drosophila is a suitable organism for investigating the relationship between gene expression and the establishment of embryonic organization. Following fertilization of the

Drosophila embryo, the nuclei undergo rapid synchronous divisions, then migrate to the periplasm and divide several more times. Cells are formed around 2.5 h after oviposition (cellular blastoderm). At about this time, transcription of the zygotic genome is also first detectable (Zalokar, 1976; Anderson and Lengyel, 1979). It is also at blastoderm that cells are initially determined (Illmensee, 1978; Wieschaus and Gehring, 1976), although morphological differences do not appear until several hours later. The determination of blastoderm cells is a function of their position in the embryo; position along the anterior-posterior axis determines segment identity, and position in the dorsal-ventral axis determines the eventual ectodermal and mesodermal layers, as well as dividing the ectoderm into neural ectoderm and dorsal and ventral epidermis (Nüsslein-Volhard, 1979). The morphogenetic movements giving rise to the germ layers occur during gastrulation (~4 h after oviposition). Visual evidence of segmentation is detectable at 5 h (Nüsslein and Wieschaus, 1980). However, the molecular nature of positional information, and of the embryonic response of the cells to this information are still unknown.

In this report we have used two-dimensional gel electrophoresis to characterize changes in protein species synthesized during early *Drosophila* embryogenesis. Of the 261 most readily detectable proteins, 68 (26%) show dramatic changes in rates of synthesis in the first 8 h of embryogenesis. To distinguish between transcriptional and translational mechanisms of control, RNA was purified from three stages and translated *in vitro*. A comparison of the *in vivo* and *in vitro* patterns demonstrated that changes in the levels of specific translatable mRNAs are correlated with many of the *in vivo* changes. The comparison also revealed several maternal mRNA species which are present in early embryos but not actively translated.

One approach to determine the role of these stage-specific proteins in embryonic development is to examine their synthesis in mutants which specifically alter pattern formation. We chose to study the *dorsal* mutation (Nüsslein-Volhard, 1979), which disrupts the dorsal-ventral polarity of the embryo, such that virtually only dorsal cuticle is differentiated. Females homozygous for the *dorsal* mutation lay eggs which form a blastoderm of normal appearance, but fail to gastrulate. While the dorsal hypoderm differentiates, the internal tissues deriving from the mesodermal and endodermal layers are absent. The *dorsal* phenotype depends strictly upon the maternal genotype, since embryos are not rescued by wild-type *dorsal* alleles from the male.

We made a thorough comparison of the protein species synthesized in wild-type and *dorsal* embryos. No qualitative differences in protein synthesis due to the *dorsal* mutation were apparent until late in embryogenesis.

Results

Analysis of proteins synthesized in vivo

To identify proteins synthesized at specific stages of early embryogenesis, ovaries, unfertilized eggs, and embryos 1, 2,

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Fig. 1. Proteins synthesized *in vivo* in embryos and ovaries labelled with [35 S]methionine and separated on two-dimensional gels. Both pH 6-8 and pH 5-7 range gels are shown, with most of the region of overlapping pH removed. The spots in the remaining overlapping region permit an evaluation of differences in exposure or migration between the gels. (a) 1 h embryos. The 30 early proteins are identified by numbers. Arrows indicate the nine discontinuous proteins (b) 3 h embryos. Early and late proteins are identified by an E or L, respectively. A2 = actin II, A3 = actin III, A1T = α_1 -tubulin; A2T = α_2 -tubulin, BIT = β_1 -tubulin. (c) 8 h embryos. The late proteins are identified by numbers. (d) Ovaries. Arrows denote proteins synthesized at higher rates in ovaries than in embryos. Early and late proteins are identified by an E or L.

Table I. Stage-specific proteins^a

Protein	pH	mol. wt. (kd)	Protein	pH	mol. wt. (kd)
E1	6.7	45	L1	6.6	64
E2	6.6	38	L2	6.5	90
E3	6.3	54	L3	6.5	86
E4	6.3	56	L4	5.3	66
E5	5.7	39	L5	5.2	100
E6	5.4	55	L6	5.8	63
E7	5.2	57	L7	5.7	64
E8	5.1	38	L8	4.7	77
E9	6.0	41	L9	6.1	34
E10	6.4	46	L10	6.0	52
E11	4.7	50	L11	6.3	50
E12	6.6	27	L12	6.7	61
E13	6.3	30	L13	6.3	50
E14	6.4	30	L14	6.1	43
E15	6.4	30	L15	6.3	40
E16	6.4	28	L16	6.5	31
E17	4.8	28	L17	5.7	58
E18	4.8	28	L18(actin I)	5.4	44
E19	6.9	60	L19	5.4	38
E20	6.7	90	L20	5.4	34
E21	7.1	28	L21(β_3 -tubulin)	5.0	51
E22	7.0	30	L22	5.9	50
E23	6.8	35	L23	4.4	51
E24	6.8	32	L24	6.7	28
E25	6.8	30	L25	6.6	21
E26	6.7	26	L26	7.0	42
E27	6.8	21	L27	7.0	39
E28	5.6	30	L28	6.8	28
E29	5.6	28	L29	5.9	48
E30	6.3	21			
D1	6.6	60			
D2	6.4	63			
D3	5.4	86			
D4	6.6	57			
D5	6.5	56			
D6	6.4	60			
D7	6.6	56			
D8	5.6	50			
D9	5.3	39			

^aThe proteins are named according to their stage-specificity, E for early, D for discontinuous, and L for late, as described in the text. The pH is the focusing pH in the first dimension, which was measured according to O'Farrell (1975). The mol. wts. were determined by running protein mol. wt. standards in the second dimension gel.

3, 4, 6 and 8 h after oviposition were labelled with [³⁵S]-methionine. Each sample was resolved by two-dimensional electrophoresis, with both 5–7 and 6–8 pH ranges in the first dimension. The patterns of protein synthesis for ovaries

and representative embryonic stages are shown in Figure 1. Spots that were of sufficient intensity for consistent scoring were chosen and their synthesis at each stage was compared. Of the 261 spots scored, 68 (26%) had pronounced differences in intensity at different embryonic stages (Table I). Almost all of the differential spots can be classified into one of three groups: early, detected at 1, 2 and 3 h, but not later; late, not detected at 1 h, but appearing later; and discontinuous, detected before and after, but not at 3 and 4 h (Table II). 30 spots were classified as early, 29 as late and nine as discontinuous.

The actins (Storti *et al.*, 1978) and tubulins (Raff *et al.*, 1982) were identified on the basis of their electrophoretic mobility, abundance and stage specificity, which are in agreement with previous reports. Actin II and actin III are made at high rates at all stages, while actin I, the muscle-specific form, is observed only in 8 h embryos. Likewise, α_1 -, α_2 - and β_1 -tubulins are synthesized at all stages, while β_3 -tubulin appears at 8 h (Raff *et al.*, 1982). Proteins E6 and E7, which differ slightly in pl and mol. wt., are synthesized at a high rate in 1 h embryos, at a lower rate in 2 h embryos, and at undetectable levels from 3 h onwards. These two proteins can be detected by staining with silver or Coomassie blue in embryo samples 1–4 h old, but not from 6 or 8 h embryos (Figure 2). Two proteins of mol. wt. 28 kd but different pIs (L24 and L28) are barely detectable before blastoderm but are synthesized at very high rates at 6 and 8 h. Of particular interest in L29, which is synthesized only at 3 and 4 h, suggesting that its function is specific to blastoderm or gastrulation.

A detailed comparison was made between the pattern of protein synthesis in ovaries and embryos. Thirty-nine proteins, identified in Figure 1, are made at much higher rates in ovaries than in embryos. Some of these are made exclusively in ovaries, while others are made in embryos at lower rates. The distribution of the embryonic proteins among the proteins synthesized in ovaries is shown in Table III. While 85% of the discontinuous and non-stage-specific proteins were also detected among the proteins synthesized in ovaries, only 59% of the early and late proteins were produced by ovaries.

Almost all proteins made in 1 h embryos are also made in unfertilized eggs. This is compatible with the finding that rates of protein synthesis are identical in fertilized and unfertilized eggs (Zalokar, 1976).

Comparison of proteins synthesized in vivo and in cell-free translation of embryonic RNA

The changing rates of protein synthesis could be explained either by: (1) changes in the mRNA population or (2) by control at the level of translation. According to the first hypothesis, early proteins would be coded for by maternal mRNAs that are not replaced by zygotic transcripts. Discontinuous proteins would be initially translated from maternal messages with short half-lives, and then reappear when sufficient message has been transcribed from the zygotic genome. Late proteins would be translated exclusively from zygotic messages. Under the translational hypothesis, certain maternal mRNAs are stored in untranslated form, and specifically recruited for translation at different stages.

To distinguish between these two hypotheses, total cellular RNA was isolated from 1 h, 3–5 h and 5–8 h embryos, the times corresponding to the three stages of protein synthesis observed *in vivo*. The RNA was translated in a rabbit reticulocyte lysate system and the translation products

separated on 5–7 and 6–8 range gels as before.

There are many spots evident on the 1 h gel that are absent or diminished on the 3–5 h gel, which parallels the decreased number of protein species detected from 1 to 3 h *in vivo* (Figure 3). A few new spots appear in the 5–8 h translation products. The patterns were analyzed in detail by identifying spots on the *in vitro* gels with mobilities identical to the *in vivo* stage-specific proteins. Spots with identical mobilities were found for 32 of the 68 stage-specific proteins, and their intensities in the translation products from the three stages were scored (Table II). The stage-specificity of the *in vivo* and *in vitro* patterns are concordant for all 17 early proteins, seven of 10 late proteins, but none of five discontinuous proteins.

The agreement of the *in vivo* and *in vitro* patterns of the early proteins indicates that the presumably maternal mRNAs coding for these proteins disappear from 1 to 3 h. We have consistently observed that the efficiency of translation in reticulocyte lysates of 1 h RNA is about twice that of 3–5 h RNA. An analysis of more precisely staged embryos has shown that the translational efficiency and the total amount of RNA per embryo steadily declines in embryos from 1 to 3 h (M. Haenlin, personal communication). Thus, the degradation of maternal mRNA offers an explanation for the decrease in protein species synthesized *in vivo* at 3 and 4 h.

The evidence for transcriptional *versus* translational control is not as conclusive for the late and discontinuous proteins. Because the scoring of minor spots may be ambiguous, the *in vivo* and *in vitro* patterns of some major proteins will be examined individually. L1, the first late protein detected *in vivo*, is present in 3–5 h *in vitro* products but not in 1 h products. The appearance of L1 at 2 h *in vivo* thus indicates early zygotic gene expression. L24 and L28, the late proteins synthesized at the highest rates *in vivo*, are absent in the 1 h *in vitro* sample, and detectable in only small amounts in the 3–5 h and 5–8 h samples. Since our unidentified spots appear in the same region of the gel at 5–8 h, the differences between the *in vivo* and *in vitro* patterns may reflect differences in either translational efficiency or post-translational processing.

The presence of major late proteins in the *in vitro* translation products of 1 h RNA could provide evidence for translational control. Two moderately abundant late proteins, L15 and L22, are not synthesized in 1 h embryos *in vivo*, yet are easily detectable in the translation products of 1 h RNA. However, the abundance of L15 is much higher in the 1 h *in vitro* products than in the *in vitro* products at later times. Both L15 and L22 are synthesized at much higher rates in ovaries than in embryos, suggesting that the messages for these proteins are translated in the oocyte, and then stored in an untranslated form in the early embryo. Several other proteins synthesized at high rates in ovaries were detected in the 1 h translation products (Figures 1 and 3).

The effect of dorsal mutation on proteins synthesized in ovaries and embryos

Since the *dorsal* mutation has a dramatic effect on morphogenesis (Nüsslein-Volhard, 1979), it might conceivably have an equally profound effect on protein synthesis. In particular, any proteins limited to mesodermal or endodermal tissues should be absent in *dorsal* embryos. We compared the proteins synthesized in *dl/dl* ovaries and in 1, 3 and 8 h embryos from *dl/dl* females with the wild-type pattern. To con-

trol for differences in genetic background, two alleles of *dorsal*, *dl¹* and *dl⁴*, as well as *dl/CyO* heterozygous controls were studied. A detailed comparison between *dl* and wild-type revealed several reproducible qualitative differences, but none of these were due to the *dorsal* mutation, and all could be attributed to irrelevant polymorphisms. As shown in Figure 4, most of the 'late' proteins are synthesized at near wild-type levels in 8 h *dorsal* embryos. The only apparent effect of *dorsal* is reduced synthesis of actin I at 8 h. To determine the effect of *dorsal* on proteins which appear later than 8 h, the proteins from 1 and 20 h Oregon R and *dl/dl* embryos were compared on Coomassie blue and silver-stained two-dimensional gels (data not shown). Actin I and actin II were present in equal amounts in the Coomassie blue stained 20 h Oregon R sample, but only actin II was detected in the 20 h *dl/dl* embryos. On the silver-stained gels, 29 new proteins appeared in the 20 h Oregon R embryos which were not present at 1 h, while only seven of these proteins appeared in the 20 h *dl/dl* embryos. Therefore, the major qualitative effects of *dorsal* on protein synthesis are apparent only after 8 h.

Discussion

We have found that the synthetic rates of 68 of the 261 proteins scored change dramatically during the first 8 h of embryogenesis. These differential proteins can be classified into three groups: early, specific to preblastoderm; late, not detected at 1 h, but appearing later; and discontinuous, synthesized before and after, but not at 3 and 4 h. A previous study (Savoini *et al.*, 1981) of *Drosophila* embryos using nearly identical methods classified only 5% of the proteins as stage-specific. We have consistently observed these stage-specific differences in repeated experiments. The two studies are in agreement that fewer proteins are synthesized at 3 or 4 h than earlier or later. This observation is consistent with the finding of Santon and Pelligrini (1981) that the rate of total protein synthesis declines almost 3-fold from 2 to 4.5 h after oviposition.

Two general hypotheses could explain the changes in proteins synthesized *in vivo*. Maternal messages could be replaced by zygotic messages containing different sequences, or different maternal messages could be recruited at specific stages from untranslated mRNP particles.

Clear evidence for translational control of specific mRNAs in early embryogenesis has been found in the surf clam (Rosenthal *et al.*, 1980) and in mice (Cascio and Wassarman, 1982). The existence of mRNA stored in the form of untranslated mRNP has been demonstrated in *Drosophila* oocytes and embryos (Goldstein, 1978). However, no differences in the mRNA sequences present in polysomes and RNP in early embryos could be detected by cDNA hybridization (Goldstein, 1978). We have shown that most of the major changes in protein synthesis occurring *in vivo* can be reproduced by *in vitro* translation of RNA from these stages, implying that changes in the translatable mRNA population are responsible for most changes in the pattern of protein synthesis. However, the presence of proteins in the *in vitro* translation products of 1 h RNA which are not synthesized in 1 h embryos suggests some form of translational control at this stage. Most of these proteins are synthesized at high rates in ovaries. Perhaps the mRNA for these proteins are translated in oocytes but remain present in early embryos in an untranslated form until they are degraded. Scherer *et al.* (1981) found by hybridization to cDNA clones that many

mRNA sequences present at 1 h have disappeared by 3–5 h, while few new sequences appear. These results correspond well with the disappearance of many proteins in the *in vitro* translation products from 1 to 3–5 h RNA samples. Extensive degradation of maternal mRNA has been reported in *Smittia* embryos (Jäckle, 1979). The degradation of part of the maternal mRNA pool near the time that embryonic

transcription begins may be important in the transition from maternal to embryonic mRNAs.

Anderson and Lengyel (1981) have shown that embryonic transcripts rapidly associate with polysomes, and that by 3.5 h 14% of the polysomal message is of embryonic origin. By 9 h, more than half of the polysomal mRNA is newly synthesized. These percentages are compatible with our findings

Table II. Patterns of stage-specific synthesis

Protein	<i>In vivo</i>								<i>In vitro</i>		
	Ovary	Unfertilized	1	2	3	4	6	8	1	3–5	5–8
E1	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++			
E2	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----	-----
E3		+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----		
E4	+++++	-----	+++++								
E5		+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----	-----
E6	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++			
E7	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++			
E8		+++++	+++++	+++++	+++++	+++++	+++++	+++++			
E9			+++++	+++++	+++++	+++++	+++++	+++++			
E10	+++++	-----	+++++								
E11			+++++	+++++	+++++	+++++	+++++	+++++			
E12	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++		
E13		+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----
E14	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++		
E15		+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----	-----
E16	+++++	-----	-----	-----	-----	-----	-----	-----	+++++		
E17	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----
E18	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----	-----
E19	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++			
E20	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++			
E21	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++		
E22	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----	
E23		+++++	+++++	+++++	+++++	+++++	+++++	+++++			
E24	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	
E25	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----
E26		+++++	+++++	+++++	+++++	+++++	+++++	+++++			
E28		+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++		
E29	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++		
E30	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----	
D1	-----	-----	-----	+++++	+++++	+++++	+++++	+++++	+++++		
D2	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++			
D3	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++			
D4	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----	-----
D5	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----	-----
D6	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----	-----
D7	+++++	-----	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----	-----
D8	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
D9	+++++		+++++	+++++	+++++	+++++	+++++	+++++			

Protein	<i>In vivo</i>								<i>In vitro</i>		
	Ovary	Unfertilized	1	2	3	4	6	8	1	3-5	5-8
L1				+++++	+++++	+++++	+++++	+++++		+++++	+++++
L2							+++++	+++++			
L3							+++++	+++++			
L4	-----		-----				+++++	+++++			
L5	-----	-----	-----					+++++			
L6	+++++						-----	+++++			
L7							+++++	+++++			
L8							+++++	+++++			
L9	+++++	-----		+++++	-----	-----	+++++	+++++			
L10				+++++	+++++	+++++	+++++	+++++		+++++	+++++
L11							+++++	+++++			
L12	+++++					-----	+++++	+++++			
L13	+++++							+++++			
L14	+++++						+++++	+++++			
L15	+++++						+++++	+++++	+++++		
L16	+++++						-----	-----		-----	-----
L17	+++++	-----	-----	-----	-----	+++++	+++++	+++++	-----	-----	-----
L18	+++++						-----	-----		-----	-----
L19							+++++	+++++			
L20							+++++	+++++			
L21								+++++			
L22	+++++						+++++	+++++	+++++	+++++	+++++
L23	-----	-----					+++++	+++++			
L24	+++++	-----		-----	-----	+++++	+++++	+++++		-----	-----
L25	-----			-----	-----	+++++	+++++	+++++	+++++	+++++	+++++
L26				+++++	+++++	+++++	+++++	+++++			
L27				+++++	+++++	+++++	+++++	+++++			
L28	-----			+++++	+++++	+++++	+++++	+++++		-----	-----
L29				+++++	+++++	+++++	+++++	+++++			

The intensities of the stage-specific spots as scored visually are denoted by '+++++', easily detectable; '-----', barely detectable; or blank, not detectable. The intensities of spots in the *in vitro* samples having the same electrophoretic mobility as the stage-specific proteins are shown in the last three columns.

Fig. 2. Region of silver-stained gels containing E6 and E7. pH 5-7 range gels were run under standard conditions with 30 unlabelled embryos aged 1 h (a) or 6 h (b). Gels were silver-stained by the method of Wray *et al.* (1981). AT = α -tubulins. BT = β_1 -tubulin.

that several embryonic-specific proteins can be detected as early as blastoderm, and 29 new proteins appear by 8 h. The mRNA sequence diversity during *Drosophila* embryogenesis has been analyzed by RNA-cDNA hybridization (Arthur *et*

Table III. Embryonic proteins synthesized in ovaries

	Early	Late	Discon- tinuous	Non-stage- specific	Total
Present in ovaries	19	16	9	163	207
Absent in ovaries	<u>11</u>	<u>13</u>	<u>0</u>	<u>30</u>	<u>54</u>
Total	30	29	9	193	261
Percentage	11.5	11.1	3.4	74.0	

al., 1979). 7.7% of the poly(A)⁺ RNA sequences present in the 3.5 h embryo are not present in the oocytes, while 2.5% of the oocyte sequences disappear during this time. However, we have found only a few proteins appearing at blastoderm, and many proteins are synthesized at 1 h but not at 4 h. This

Fig. 3. Translation products of total cellular RNA separated on pH 5–7 range gels. 200 000 c.p.m. of TCA-precipitable counts were applied to each gel. Fluorographic exposure was 7 days. (A) Translation products of 1 h RNA; (B) translation products of 3–5 h RNA. E12, L1, L15, L22 and L24 are stage-specific proteins. Proteins labelled with a 'O' are synthesized *in vivo* at higher rates in ovaries than in embryos (see Figure 1). 'E' denotes background endogenous to the reticulocyte lysate.

Fig. 4. Proteins synthesized *in vivo* in 1 h and 8 h dorsal embryos. Embryos were collected from a cage containing males and females homozygous for the second chromosome *dl cn sca*. Labeling, sample preparation and two-dimensional electrophoresis on pH 5–7 range gels were as described in Materials and methods. (A) 1 h embryos. (B) 8 h embryos. The most readily detectable 'late' proteins are designated by numbers. 18 = actin 1, 21 = β_3 -tubulin.

apparent discrepancy may be explained by the fact that RNA-cDNA hybridization measures all abundance classes of RNA, while two-dimensional gels detect only moderately-abundant and abundant proteins.

We have detected many proteins that are made predominantly from either maternal or zygotic mRNAs. The synthesis

of particular proteins at specific times suggests but does not prove that these proteins are required for only that developmental stage. Maternal mRNA may simply be an efficient means of providing the embryo with 'housekeeping' proteins. However, proteins E6 and E7 are apparently involved in processes specific to the early embryo since they disappear from

the embryos after 4 h. Although we have classified all zygotic-specific proteins as 'late', different late proteins appear at particular stages. L1 appears at 2 h, and is the first zygotic protein detectable *in vivo* and *in vitro*. The early appearance of an apparently different blastoderm-specific protein has been reported by Gutzeit (1980). The 28-kD proteins L24 and L28 are synthesized in small amounts at 3 and 4 h, but are major products at 6 and 8 h. Actin I and β_3 -tubulin appear at 8 h, marking the onset of cellular differentiation. A comparison of silver-stained gels from 1 and 8 h embryos revealed no qualitative differences except for the disappearance of E6 and E7. When the comparison was extended to 20 h embryos, many qualitative differences were seen, primarily the appearance of many new proteins in the 20 h embryos.

Cells are initially determined at blastoderm to follow particular developmental pathways, but it is not known how early determination is expressed at the level of moderately abundant mRNAs or proteins. After gastrulation at 4 h the embryonic germ layers of ectoderm, mesoderm and endoderm are established. We have compared the proteins synthesized in wild-type and *dorsal* embryos, in which the mesodermal and endodermal layers are not produced (Nüsslein-Volhard, 1979). No differences in the pattern of protein synthesis due to the *dorsal* mutation could be detected except for reduced synthesis of actin I at 8 h. In differentiating *Drosophila* myoblasts, actin I is the first muscle-specific protein synthesized (Storti *et al.*, 1978). Therefore, the absence of mesodermal and endodermal tissues in *dorsal* embryos is not manifested at the level of moderately abundant proteins until the onset of differentiation. It remains to be seen whether any of the zygotic proteins appearing before 8 h are the result of determinative events, or whether they are synthesized in all embryonic cells.

Materials and methods

Fly strains

Oregon R was the standard wild-type strain. Two alleles of *dl* in differently marked stocks, *dl¹ cn sca/CyO* and *dl²/CyO* (obtained from C. Nüsslein), were used to control for genetic background. To obtain dorsal embryos, homozygous *dl* females and males were manually segregated from heterozygotes on the basis of the *Curly* phenotype, then mass-mated. In one experiment, *dl* homozygotes were produced by growing at the restrictive temperature (26°C) a stock containing a dominant temperature-sensitive lethal on the second chromosome balancer (*dl¹ cn sca/CyO DTS-100*).

Labeling of embryos

Flies were grown at 25°C in population cages, and fed with yeast-paste smeared on grape-agar plates. After a 1 h precollection, eggs were collected for 1 h intervals and aged at 25°C for the following times before labeling (measured from the midpoint of collection to the beginning of labeling): 1 h (nuclear multiplication), 2 h (syncytial blastoderm), 3 h (cellular blastoderm), 4 h (gastrulation), 6 h (mesodermal segmentation) and 8 h. Unfertilized eggs were collected from virgin Oregon R females and labeled at 1 h. Embryos were dechorionated in 3% sodium hypochlorite for 2 min, then permeabilized in octane for 3 min according to Limbourg and Zalokar (1973), and rinsed thoroughly in Insect Ringers (130 mM NaCl, 4.6 mM KCl, and 1.5 mM CaCl₂). The permeabilized embryos were labeled for 30 min at 25°C in egg incubation medium (Limbourg and Zalokar, 1973) containing 1 mCi/ml [³⁵S]-methionine (New England Nuclear, ~1000 Ci/mmol). Incorporation of label into TCA-precipitable counts declined from a maximum of ~5000 c.p.m./embryo at 1 h to ~1000 c.p.m./embryo at 8 h. Ovaries were dissected from 3-day old Oregon R females and incubated for 30 min in Robbs medium (Robb, 1969) without cold methionine containing 0.5 mCi/ml [³⁵S]-methionine.

After labeling, the embryos or ovaries were homogenized with either a 50 μ l Hamilton syringe or a small glass homogenizer, in 50 mM Tris-HCl pH 7.6, 0.3% SDS, 1% β -mercaptoethanol. A 1/10 volume aliquot of 1 mg/ml DNase I, 500 μ g/ml RNase A, 0.5 M Tris-HCl pH 7.0, 50 mM MgCl₂ was

added to the homogenate which was then incubated on ice for 1 min. The samples were frozen in liquid nitrogen, lyophilized, and dissolved in the sample buffer of Garrels (1979), containing 9.95 M urea, 4.0% NP-40, 2% ampholines 6–8 range and 100 mM dithiothreitol.

Two-dimensional gel electrophoresis

Two-dimensional gels were run according to O'Farrell (1975) with minor modifications. The number of volt-hours for optimal resolution in the first dimension was determined empirically for each pH range. First dimension gels containing 5–7 range ampholines were run in 13 cm length glass tubes for 8000 volt-hours. First dimension gels with 6–8 range ampholines were run in 16 cm length glass tubes for 18 000 volt-hours. 2 cm of the acidic end of the 6–8 range gels was removed before application of the gels to the second dimension. The second dimension gels were 10% acrylamide. All gels were stained with Coomassie blue, then processed for fluorography according to Laskey and Mills (1975). Usually each gel was loaded with 100 000 c.p.m. of TCA-precipitable counts and exposed to Kodak X-omat AR-5 film for 10 days. Films were analyzed by assigning numbers and X- and Y-coordinates to all readily identifiable spots. The intensity of these spots was evaluated visually on each gel in a series. Gels run at different times showed some variability in migration patterns, but were sufficiently similar for unambiguous matching of spots between series. All of the reported results were verified in at least two independent experiments.

Silver staining of gels was performed according to Wray *et al.* (1981).

RNA extraction and *in vitro* translation

Total cellular RNA was extracted from frozen embryos by LiCl-urea precipitation from cell homogenates (Auffray and Rougeon, 1980) as modified by LeMeur *et al.* (1980). RNA was translated at a concentration of 200 μ g/ml in rabbit reticulocyte lysates (New England Nuclear) for 1 h at 37°C. Incorporation into TCA-precipitable counts was 10–20 times above background without added RNA. Samples were processed and electrophoresed in the same manner as the *in vivo* samples.

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