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Protein Synthesis in Embryonic Tissues During Mouse Postimplantation Development

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Mouse embryos of the NMRI strain between the 7th and 9th day of gestation were isolated from the uterus and dissected into the various tissue derivatives in order to investigate newly synthesized proteins during morphogenesis. The day 7 embryo was fragmented into trophoblast and ectoplacental cone, distal and proximal endoderm, extraembryonic and embryonic ectoderm. The day 8 and day 9 embryos were divided into trophoblast and placental anlage, yolk sac, amnion, and allantois, as well as cranial, central, and caudal embryonic tissue. The intact embryos were incubated in Dulbecco's minimum essential medium in the presence of ^{35}S-methionine for 4 h, then dissected into the various fragments, and further processed for two-dimensional gel electrophoresis. Protein synthesis of the isolated tissue derivatives was analyzed and compared for the three developmental stages. Concerning the proteins with isoelectric points in the range of 4.5 to 8.0 and molecular weight ratio (M_r) values between 20,000 and 200,000, we found several significant quantitative and qualitative differences in the various tissue fragments. In addition, we observed further quantitative and qualitative differences in protein synthesis during the postimplantation period investigated. We propose that the differences reflect some of the cell lineage- and developmental stage-specific changes in gene expression during early mammalian differentiation.

Key words: cell lineage, tissue separation, gene expression, 2-D gels, mammalian embryology

In the mouse the initial phase of development takes about 5 days from fertilization to the end of preimplantation. During this period, certain cell lineages are already being established for further differentiation [1–3]. Following implantation, the embryo is growing very rapidly, and its mass is increasing logarithmically between the 7th and 9th day of gestation [4,5]. At that stage in development, organogenesis is also initiated and organs derived from the three germ layers are being formed [6,7]. These dramatic morphogenetic processes must be paralleled by major biochemical changes on the transcriptional as well as on the translational level [reviewed in 8].

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The preimplantation period has already been well studied concerning RNA synthesis [9,10], protein synthesis [11–14], as well as synthesis of specific gene products [15–20]. One of the reasons for the increasing knowledge about gene activity during early embryogenesis is that embryos at this stage are easily accessible to the investigator by simply flushing them from the genital tract. It was found that very significant changes in protein synthesis occur during the two- to eight-cell stage, indicating early embryonic gene expression, whereas the protein pattern during late preimplantation remains rather similar [reviewed in 21].

The early postimplantation period, however, is more difficult to investigate, because the small embryo has to be dissected from the uterus. Information about gene activity in embryos following implantation or after culture in vitro is still rather rudimentary and has so far been related to proteins [22–25], antigens [26,27], oncogenes [28–36], homeobox genes [37–43], mutations [44,45], or cytoskeletal elements [46–49]. Two-dimensional (2-D) polyacrylamide gel electrophoresis seems to be an appropriate experimental tool to gain further insight into these biological events coinciding with gene expression and morphogenesis.

In the paper presented here, we studied protein synthesis between days 7 and 9 of gestation. By dissecting the embryo into its different cell layers, we examined whether or not tissue- and developmental stage-specific proteins already occur during early postimplantation development. Temporal and spatial changes in the patterns of proteins newly synthesized may be related to the morphogenetic processes observed and, eventually, to certain genes responsible for cell lineage specificity and differentiation. Part of this work has already been presented in abstract form [50].

MATERIALS AND METHODS

Embryonic Tissues

Mice from the NMRI strain were purchased from the Füllinsdorf Institute for Biomedical Research (BL, Switzerland) and bred in our animal colony. Postimplantation embryos were obtained from spontaneous matings. The day of the copulation plug was designated as day 1 of gestation. Embryos at days 7, 8, and 9 of gestation were dissected from the uterus and washed in phosphate-buffered saline (PBS). Then they were placed in drops (50 μl) of Dulbecco's modified Eagle's medium (DMEM) containing ^{35}S-methionine (577 MBq/ml, 44 TBq/mmol, Radiochemical Centre, Amersham, Buckinghamshire, UK) under paraffin oil (Merck, Darmstadt, FRG) at 37°C in a humidified incubator for 4 h. Subsequently, the embryos were dissected in Hanks's calcium- and magnesium-free balanced salt solution (Hanks's CMF-BSS; GIBCO, Grand Island, NY, USA) using tungsten needles and watchmaker forceps under a stereomicroscope as has been described [5,51]. For each series of experiments, about 20 embryos at day 7 were fragmented into trophoblast, ectoplacental cone, endoderm (visceral and parietal taken together), extraembryonic and embryonic ectoderm. For each series of experiments, about 20 embryos at day 8 were divided into trophoblast and placental anlage, yolk sac, amnion, allantois, as well as cranial, central, and caudal embryonic tissue (Fig. 1). At day 9, for each series of experiments, about 10 embryos were dissected into the various tissues, as done with the day 8 embryos, except for the central embryonic part, from which only the heart was taken. During in vitro incubation of the entire embryos, their hearts were beating throughout this period. Although great care was taken generally during mechanical dissection, occasional small contamination

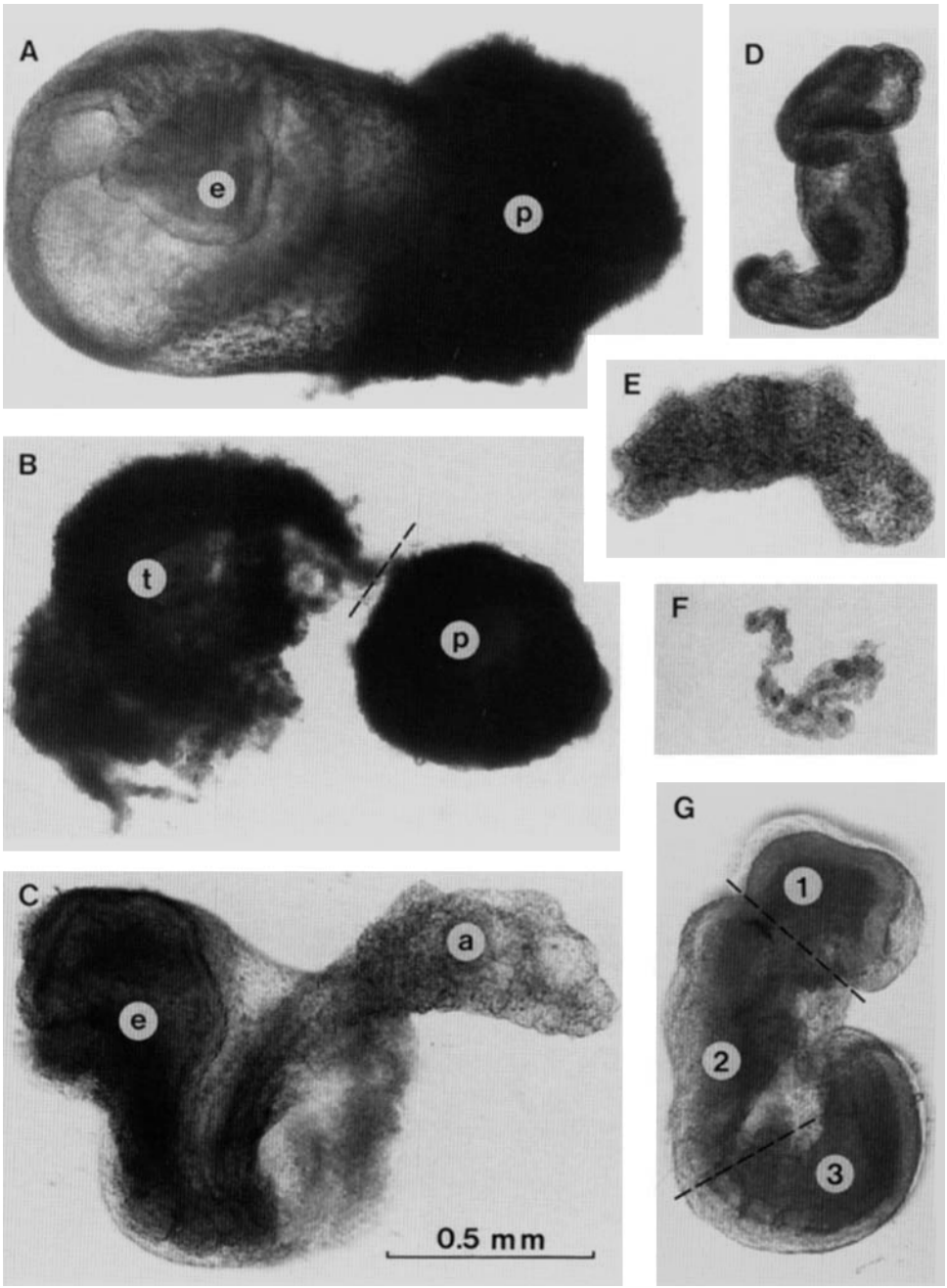


Fig. 1. Mouse embryo at day 8 of development. **A:** Embryo (e) with ectoplacental cone (p) after its isolation from the uterus; **B:** Trophoblast (t) and ectoplacental cone (p); **C:** Embryo within amnion (e) and allantois (a); **D:** Yolk sac; **E:** Allantois; **F:** Amnion; and **G:** Embryo prior to dissection into cranial (1), central (2), and caudal (3) regions.

between tissues could not be excluded, e.g., between trophoblast and ectoplacental cone or placental anlage, between extraembryonic and embryonic ectoderm, or between the embryo proper and part of the amnion.

The isolated fragments were added directly to 50 μl of lysis buffer (9.5 M urea, 2% w/v Nonidet P40, 5.5% w/v ampholytes pH 3.5–10, 2% v/v 2-mercaptoethanol). Samples of each obtained about 10 or 20 embryos and were stored at −80°C.

Gel Electrophoresis

For the first-dimensional separation, isoelectric focusing (IEF) was applied according to established procedures [52] with modifications [53]. The 10 μl samples were loaded onto the IEF gels. For the second-dimensional separation, sodium dodecyl sulphate (SDS) slab gel electrophoresis was carried out on homogeneous gels (10% w/v T, 2.6% w/v C) with stacking gels (3% w/v T, 2.6% w/v C) on top of the separating gels, as described previously [54]. More than 200 gels were processed for fluorography according to standard procedures [55,56] and exposed to preflashed x-ray films (Kodak XAR 5) at −80°C [57]. Samples from the same series of experiments (i.e., embryo preparations from the same day) were repeated two to five times. Samples from different series of experiments (i.e., embryo preparations from different days) were carried out four to eight times. For comparing and localizing protein spots from different tissues and developmental stages, the corresponding x-ray films were superimposed upon each other. For further analysis of protein identification, we have sent original gels to Professor J. Celis (Aarhus protein data bank).

RESULTS

Protein Synthesis of the Day 7 Embryo

The following tissues were analyzed for newly synthesized proteins: trophoblast, ectoplacental cone, visceral and parietal endoderm together, extraembryonic and embryonic ectoderm (Fig. 2).

Representative and reproducible results obtained from 2-D gel electrophoresis are shown in Figure 3. By comparing their protein patterns, it is found that extraembryonic and embryonic ectoderm are quite similar; the same holds true for trophoblast and ectoplacental cone. Visceral and parietal endoderm, on the other hand, show differences from the other tissues.

In particular, we focused on several proteins (numbered 1–24, see Table I), including the triple spots 12, 13, 23, and four groups of proteins (14–17) concerning their presence at day 7 and followed them through day 8 and day 9. In addition, we traced back five protein spots from day 8 to day 7 (29, 30, 31, 39, 40).

Protein Synthesis of the Day 8 Embryo

We analyzed the tissue fragments, such as trophoblast, placental anlage, yolk sac, allantois, amnion, and three regions of embryonic tissue (Fig. 4). The 2-D gel electrophoresis (Fig. 5) reveals, in general, similarities in the protein pattern of trophoblast and placental anlage, although the latter shows several additional proteins. Similarities are also seen between allantois and amnion, whereas the yolk sac differs from the other tissues and shows fewer proteins. With respect to embryonic tissues, the central and caudal regions are very similar, but the cranial part shows a more complex pattern.

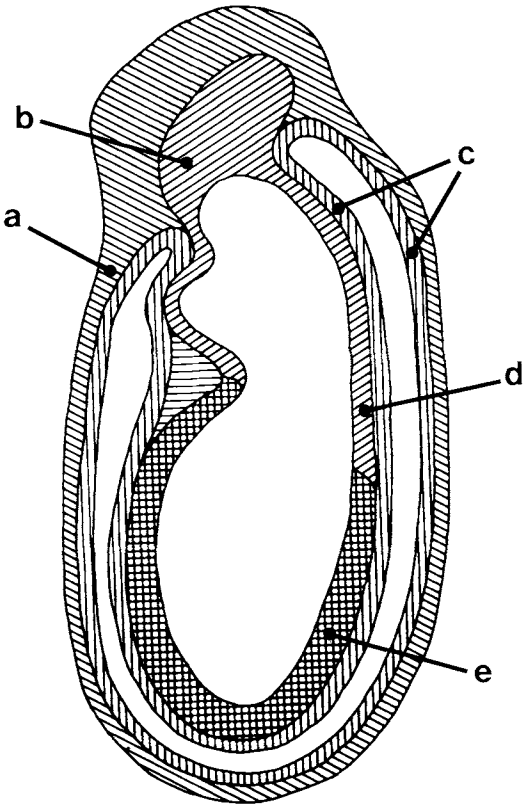


Fig. 2. Schematic drawing of a sagittal section of mouse embryo at day 7: Trophoblast (a); ectoplacental cone (b); visceral and parietal endoderm (c); extraembryonic (d) and embryonic (e) ectoderm.

We selected again several proteins (numbered 25–41; see Table II), including four groups of proteins (16, 17, 26, 38), and followed them in the different tissues and at stage 7 and 9 as well. In addition, we examined the behaviour of those proteins already appearing at day 7 (1–24) or that are present at day 9 (42–53).

Protein Synthesis of the Day 9 Embryo

Finally, we studied the newly synthesized proteins in tissues such as trophoblast, placental anlage, yolk sac, allantois, amnion, cranial embryonic tissue, heart and caudal embryonic tissue (Fig. 6). A general comparison of the protein patterns of the different tissues has shown that trophoblast and placental anlage are quite similar. The yolk sac differs from the other tissues and shows significantly less proteins, as does the allantois and to some extent the amnion. For the embryonic tissues, we have enlarged two areas for detailed comparison and in relation to the other tissues.

Instead, of the entire central embryonic region (as for the day 8 embryo), we have taken only the heart anlage for our study of newly synthesized proteins. Its pattern in the pH 5 region (Gy) is more complex when compared with the cranial and caudal tissues and shows additional proteins (45, 51, 52, 53) in the pH 7 region (Gx).

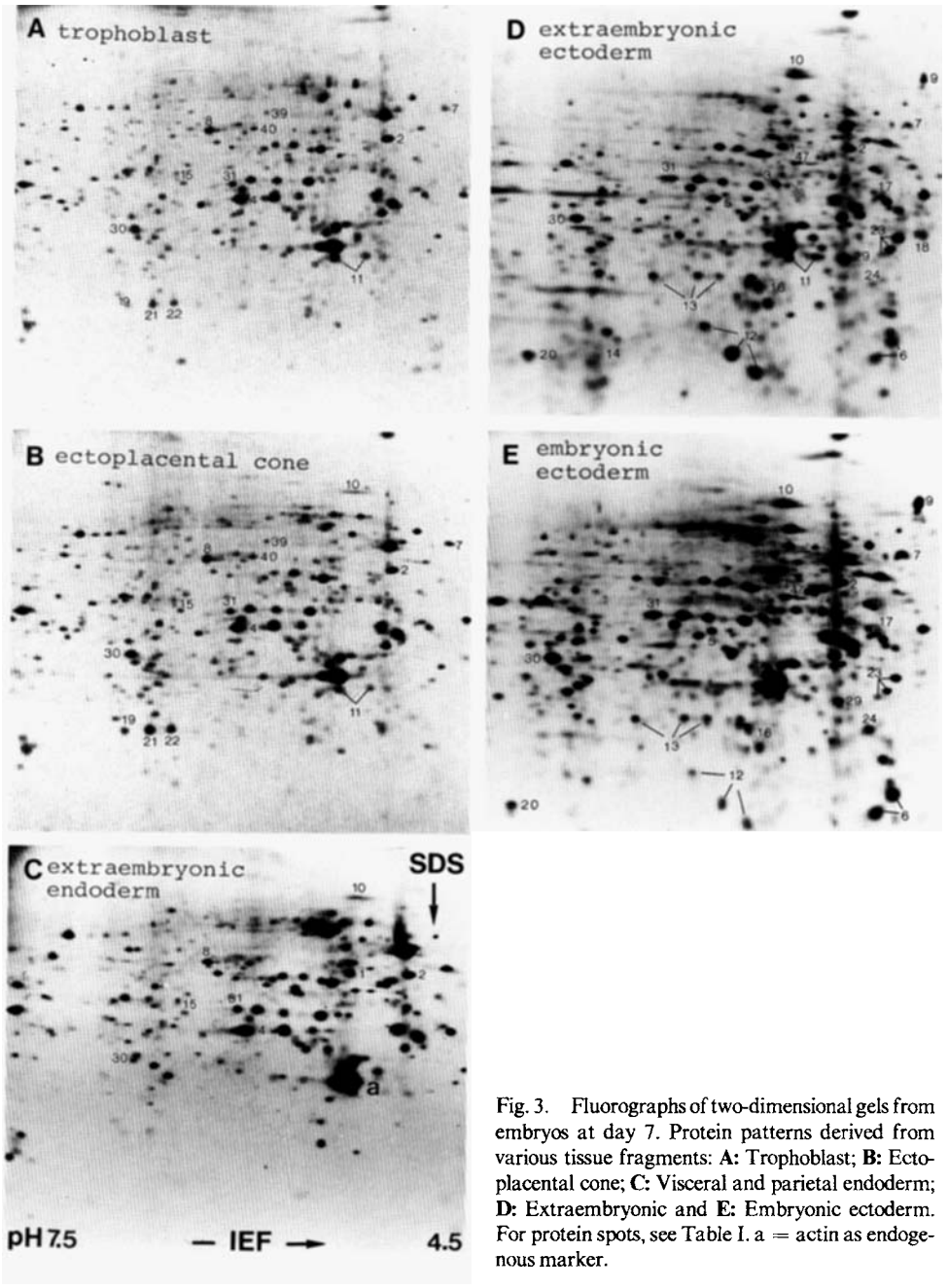


Fig. 3. Fluorographs of two-dimensional gels from embryos at day 7. Protein patterns derived from various tissue fragments: **A:** Trophoblast; **B:** Ectoplacental cone; **C:** Visceral and parietal endoderm; **D:** Extraembryonic and **E:** Embryonic ectoderm. For protein spots, see Table I. a = actin as endogenous marker.

The proteins designated to the various tissues of the day 9 embryo are numbered 42 to 53. In addition, we also examined several proteins corresponding to embryos at day 7 and 8 (see Table III).

Lineage-Related Proteins in Tissues from Day 7 to Day 9 Embryos

We studied the protein patterns of the various tissues and followed them through subsequent developmental stages of early postimplantation. Trophoblast, ectoplacental

TABLE I. Proteins Numbered for the Various Tissues of Day 7 Embryos*

Spots of proteins	Tissue type				
	Trophoblast A	Ectoplacental cone B	Visceral and parietal endoderm C	Extraembryonic ectoderm D	Embryonic ectoderm E
1	−	−	+	+	+
2	+	+	+	+	+
3	−	−	−	(+)	+
4	+	+	+	−	−
5	−	−	−	(+)	+
6	−	−	−	+	+
7	+	+	−	+	+
8	+	+	+	−	−
9	−	−	−	+	+
10	−	(+)	(+)	+	+
11	+	+	−	+	−
12	−	−	−	+	(+)
13	−	−	−	+	+
14	−	−	−	+	−
15	+	+	+	−	−
16	−	−	−	+	+
17	−	−	−	+	+
18	−	−	−	+	−
19	(+)	+	−	−	−
20	−	−	−	+	+
21	+	+	−	−	−
22	+	+	−	−	−
23	−	−	−	+	+
24	−	−	−	(+)	+
29	−	−	−	+	+
30	−	−	−	+	+
31	+	+	+	+	+
39	+	+	−	−	−
40	+	+	−	−	−
47	−	−	−	+	+

14, 15, 16, 17 are groups of proteins. + = well expressed; (+) = moderately expressed; − = absent or very weakly expressed.

*For numbers 1–24, see Figure 3; for numbers 29–47, see Figure 5.

cone, and placental anlage were taken as derivatives of a common cell lineage. Other extraembryonic tissues (ectoderm and endoderm), yolk sac, allantois, and amnion were also investigated. Tissues originating from embryonic ectoderm, endoderm, and mesoderm were grouped as embryonic tissues (Table IV). By comparing the pattern of proteins newly synthesized, we focused on 42 proteins or groups of proteins in developmentally related tissues that either persist, newly appear, or disappear during the entire period analyzed (see arrows). Quantitative modulation of protein synthesis (as deduced from the spot density) is sometimes difficult to evaluate because variations in radioactive intensity of protein spots may occur between different gels. Variable amounts of a protein can also be synthesized differently in the various tissues of a given developmental stage

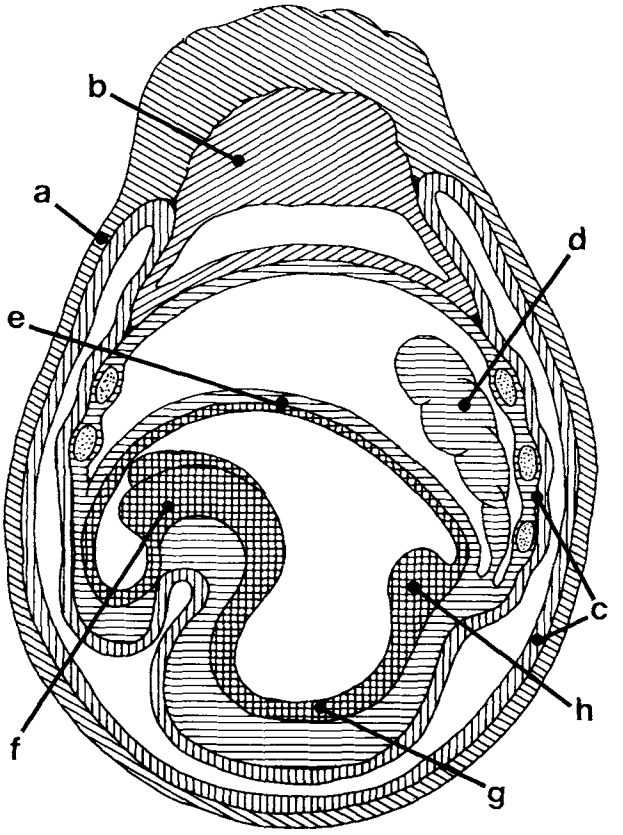


Fig. 4. Schematic drawing of a sagittal section of mouse embryo at day 8: trophoblast (a); placental anlage (b); visceral and parietal yolk sac (c); allantois (d); amnion (e); cranial (f); central (g); and caudal (h) embryonic tissue.

(6, 12, 16, 17, 21, 22, 39, 40, 54; for other examples of this type of modulation, see Tables I–III and compare horizontally the symbols + and (+) for a given protein).

Stage-Related Proteins in Tissues of the Day 7, Day 8, and Day 9 Embryo

The various tissues from embryos at days 7, 8, and 9 were analyzed for newly synthesized proteins and compared with each other to define those proteins or groups of proteins that were detectable only at a particular developmental stage.

In embryonic tissues at day 7, we observed proteins that were not seen or that were very weakly expressed during further development (3, 14, 15; see Fig. 3 and Table IV). At day 8, some other proteins were appearing on the gels that could not be detected at days 7 or 9 (26, 27, 32, 35, 37, 38, 41; see Fig. 5 and Table IV). Groups of proteins 26, 38, and spot 37 were seen only in placental anlage, spot 41 in trophoblast, spot 27 in both, and spot 35 only in the frontal part of the embryo, whereas spot 32 appeared in several tissues. In tissues of day 9 embryos, again other new proteins could be detected (42–44, 46, 48–53; see Fig. 7 and Table IV). We do not know, of course, if these proteins or some

of them are seen only in day 9 embryos or if they may persist through later development not yet analyzed. Spots 42, 43, 49, and 50 appeared in trophoblast and placental anlage. The group of proteins 46 and spot 48 were found in the yolk sac. Spots 51, 52, and 53 were specific for the heart, as well as spot 45, which had already appeared only in the middle part of the day 8 embryo.

DISCUSSION

In this paper we have described protein synthesis in mouse embryos during early postimplantation development from day 7 to day 9 of gestation. Such a descriptive approach is required as initial basis for future studies aiming to correlate particular proteins to their genes. The essential goal of our studies presented here was to compare, on the one hand, protein synthesis of different tissues of a particular developmental stage and, on the other hand, to follow protein synthesis of a given tissue through subsequent developmental stages. In other words, can we detect newly synthesized proteins that are tissue- or stage-specific?

In addition, we wanted to find out if we can detect proteins that may be regulated or modulated in their activities by comparing the patterns obtained from the various tissues at different developmental stages. Finally, are there proteins that can be considered cell lineage-specific?

First, we should emphasize that with the particular techniques used, we have resolved on our gels about 500 (about $1/20$ of the estimated protein population) newly synthesized proteins that range between 4.5 and 8.0 in their isoelectric points and 20,000 to 200,000 in their molecular weight ratios (M_r) values. The majority of these proteins is found in all different tissues and during the developmental period analyzed and can mostly be regarded as housekeeping proteins. Among those proteins we have considered about one fifth of them for our analysis and finally selected 54 proteins or groups of proteins for a more detailed study for which over 200 gels have served as basis for documentation.

When compared with the overall protein pattern of the day 8 embryo recently published [25] (Fig. 1), the pattern of protein synthesis is quite similar in qualitative and quantitative aspects. A detailed comparison, of course, is hampered by the fact that these investigators did not fragment the embryos into the various tissues. This is most likely the reason for their statement that "the gels consistently and reproducibly resolved 600 to 800 polypeptides, almost all of which showed no quantitative or qualitative differences between 8 and 10 days," except for one particular protein. This globin spot, however, can not be seen in our studies since its M_r ranges below the resolution limits of our gels. On the other hand, having seen pronounced and reproducible changes in protein patterns derived from the various tissues during day 7 to day 9 of gestation, we would like to suggest that analysis of total embryos will not reveal subtle spatial and temporal changes in protein synthesis. Indeed, the degree of precision in tissue separation carried out in our studies is a necessary prerequisite.

Referring to another previously published study [23] (Figs. 3, 4), we find that our protein patterns presented here differ quite significantly from those obtained from embryos cultured in vitro up to the 10th equivalent gestation day. Relevant to this discrepancy, we would like to quote the authors themselves: "It is important to remember

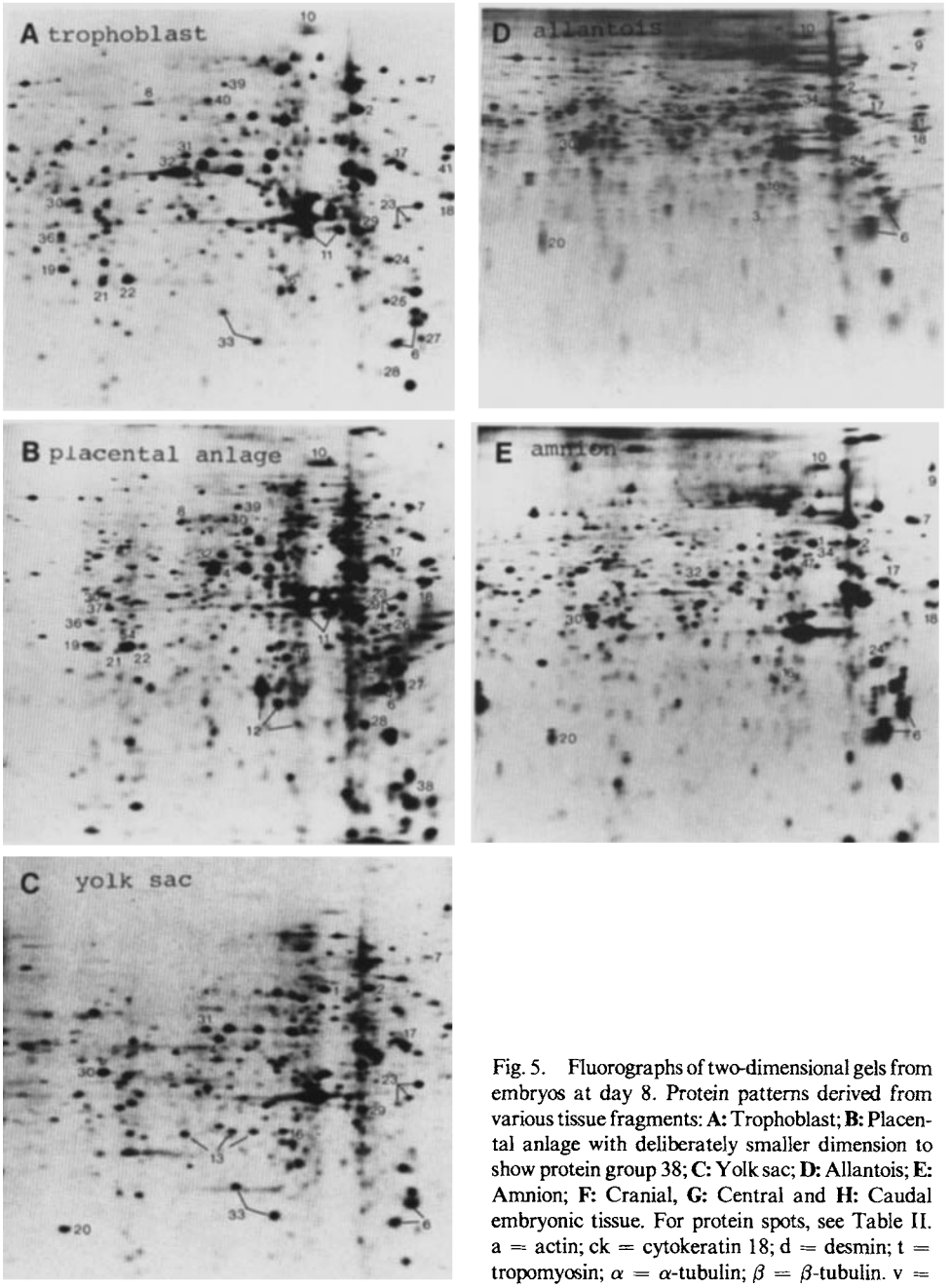
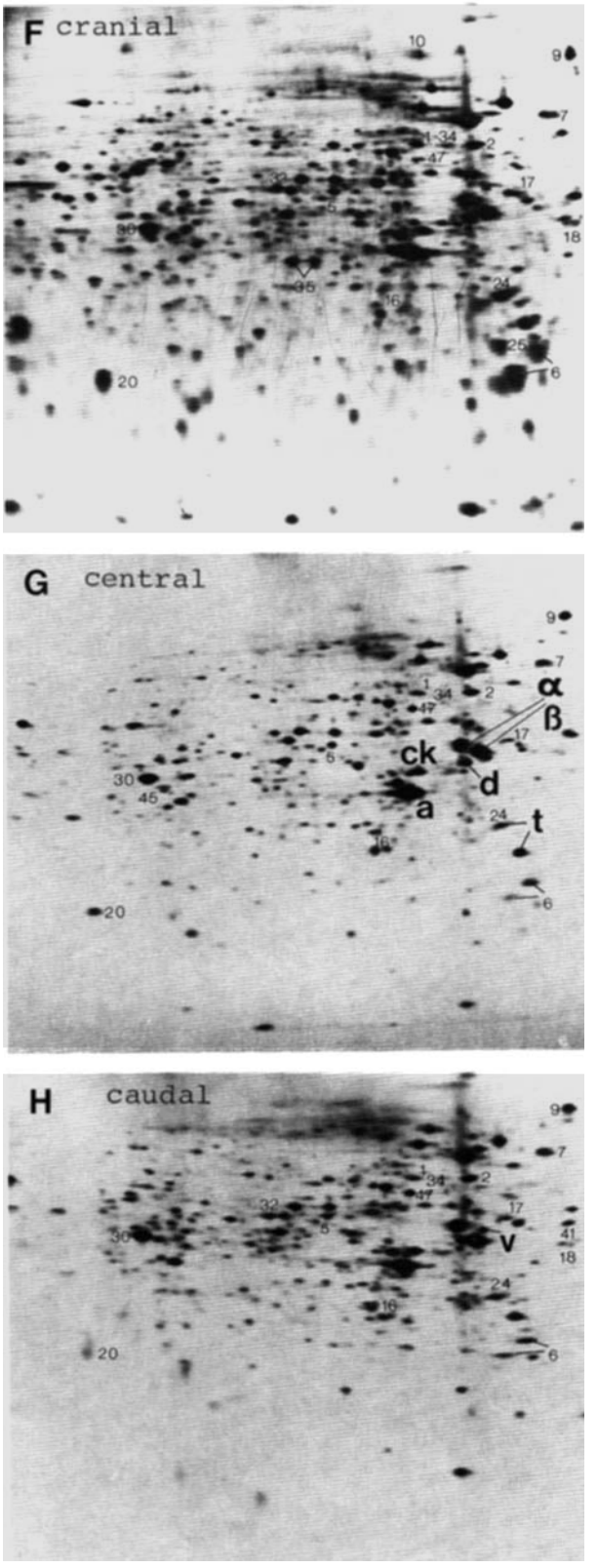


Fig. 5. Fluorographs of two-dimensional gels from embryos at day 8. Protein patterns derived from various tissue fragments: **A:** Trophoblast; **B:** Placental anlage with deliberately smaller dimension to show protein group 38; **C:** Yolk sac; **D:** Allantois; **E:** Amnion; **F:** Cranial, **G:** Central and **H:** Caudal embryonic tissue. For protein spots, see Table II. a = actin; ck = cytokeratin 18; d = desmin; t = tropomyosin; α = α-tubulin; β = β-tubulin. v = vimentin.


F cranial
10
9
7
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2
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17
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45
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16
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6
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G central
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7
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ck
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30
45
d
a
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t
16
6
20
H caudal
9
7
1
34
2
32
17
17
30
5
v
41
18
24
16
20
6

Figure 5F–H

TABLE II. Proteins Numbered for the Various Tissues of Day 8 Embryos*

Spots of proteins	Tissue type							
	Trophoblast A	Placental anlage B	Yolk sac C	Allantois D	Amnion E	Cranial embryonic tissue F	Central embryonic tissue G	Caudal embryonic tissue H
25	+	+	–	–	–	+	–	–
26	–	+	–	–	–	–	–	–
27	(+)	+	–	–	–	–	–	–
28	(+)	+	–	–	–	–	–	–
29	+	+	+	–	–	–	–	–
30	–	–	–	+	+	+	+	+
31	+	–	+	–	–	–	+	–
32	+	+	–	+	+	+	–	+
33	+	–	+	–	–	–	–	–
34	–	–	–	+	+	+	(+)	(+)
35	–	–	–	–	–	+	–	–
36	+	+	–	–	–	–	–	–
37	–	+	–	–	–	–	–	–
38	–	+	–	–	–	–	–	–
39	+	+	–	–	–	–	–	–
40	+	+	–	–	–	–	–	–
41	+	–	–	+	–	–	–	+
1	–	–	+	–	+	+	+	+
2	+	+	+	+	+	+	+	+
4	(+)	+	(+)	–	–	–	–	–
5	–	–	–	–	–	+	+	+
6	+	+	+	+	+	+	(+)	(+)
7	+	+	–	+	+	+	+	+
8	+	+	–	–	–	–	–	–
9	–	–	–	+	+	+	+	+
10	+	+	–	+	+	+	–	–
11	+	+	–	–	–	–	–	–
12	–	+	–	–	–	–	–	–
13	–	–	+	–	–	–	–	–
16	(+)	+	(+)	(+)	(+)	+	(+)	(+)
17	+	+	+	(+)	(+)	+	(+)	(+)
18	+	+	–	+	+	+	–	(+)
19	+	+	–	–	–	–	–	–
20	(+)	–	+	+	+	+	+	(+)
21	+	+	–	–	–	–	–	–
22	+	+	–	–	–	–	–	–
23	+	+	+	–	–	–	–	–
24	+	+	–	+	+	+	+	+
45	–	–	–	–	–	–	+	–
47	–	–	–	–	(+)	(+)	+	+
54	–	+	–	–	–	–	–	–

16, 17, 26, 38 are groups of proteins. + = well expressed; (+) = moderately expressed; – = absent or very weakly expressed.

*For numbers 25–41, see Figure 5; for numbers 1–24, see Figure 3. For numbers 45–54, see Figure 7.

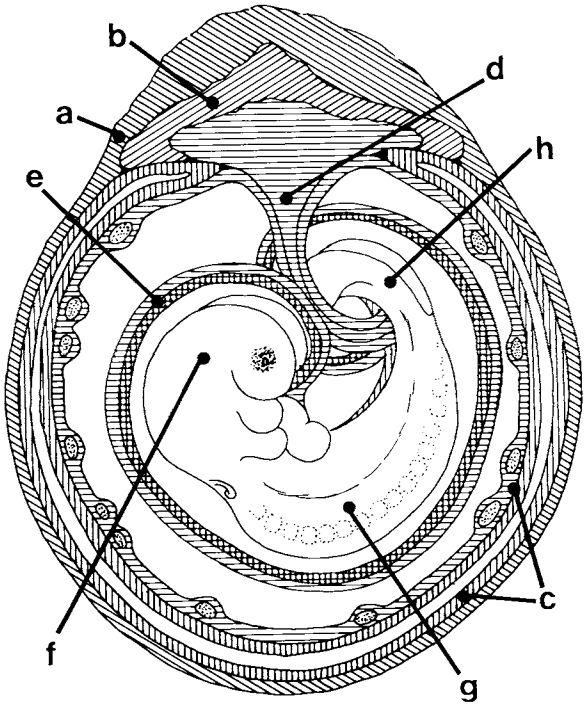

Fig. 6. Schematic drawing of a sagittal section of mouse embryo at day 9: trophoblast (a); placental anlage (b); visceral and parietal yolk sac (c); allantois (d); amnion (e); cranial (f), central (g) and caudal (h) embryonic tissue. From the central region, the heart was isolated and analyzed separately (as Gy in Fig. 7).

that this is an unnatural environment and that this probably means that all the changes occurring in vivo will not occur in vitro."

We would like to discuss now some features that have emerged from the study presented here. When comparing, in general, protein synthesis in the different embryonic tissues at day 7, it is quite obvious that the pattern of extraembryonic and embryonic ectoderm is more complex than that obtained from the other tissues. Trophoblast and ectoplacental cone show a very similar pattern and reveal their common cell lineage [24].

At day 8, the most conspicuous changes occur in the placental anlage when compared with the ectoplacental cone and extraembryonic ectoderm at day 7, from which it is originating [6]. Several new proteins and groups of proteins appear and may be linked to early placental differentiation, as proposed for another placenta-related gene product [58]. The same, although less pronounced, holds true for the yolk sac when compared with the extraembryonic endoderm of the day 7 embryo from which it partially derived [2]. Concerning the embryo proper, its protein synthesis is more complex in the cranial region than in the middle and caudal part. During neurulation, important morphogenetic processes take place in this region [6]. Putative neural-specific gene products have been detected [29,31,59,60], but their functional role remains to be uncovered.

TABLE III. Proteins Numbered for the Various Tissues of Day 9 Embryos*

Spots of proteins	Tissue type: Trophoblast A	Placental anlage B	Yolk sac C	Allantois D	Amnion E	Cranial embryonic tissue Fx, Fy	Heart Gx, Gy	Caudal embryonic tissue Hx, Hy
42	+	(+)	–	–	–	–	–	–
43	+	(+)	–	–	–	–	–	–
44	–	–	–	–	–	+	–	+
45	–	–	–	–	–	–	+	–
46	–	–	+	–	–	–	–	–
47	–	–	–	+	+	+	+	+
48	–	–	+	–	–	–	–	–
49	+	(+)	–	–	–	–	–	–
50	+	(+)	–	–	–	–	–	–
51	–	–	–	–	–	–	+	–
52	–	–	–	–	–	–	+	–
53	–	–	–	–	–	–	+	–
54	–	(+)	–	–	–	–	–	–
1	–	–	–	+	+	+	+	+
2	+	+	–	+	+	+	+	+
4	+	+	+	–	–	–	–	–
5	–	–	–	–	–	(+)	+	+
6	+	+	–	–	+	nd	nd	nd
7	+	+	–	+	+	+	+	+
8	+	(+)	–	–	–	–	–	–
9	–	–	–	+	+	+	+	+
11	+	+	–	–	–	–	–	–
13	–	–	+	–	–	nd	nd	nd
17	+	+	–	–	+	(+)	(+)	(+)
18	+	+	–	–	(+)	–	–	–
19	+	(+)	–	–	–	–	–	–
20	–	–	+	–	+	nd	nd	nd
21	+	(+)	–	–	–	–	–	–
22	+	(+)	–	–	–	–	–	–
24	+	+	–	–	–	nd	nd	nd
25	+	+	–	–	–	nd	nd	nd
28	+	+	–	–	–	–	–	–
29	+	+	–	–	–	–	–	–
30	–	–	–	–	–	+	+	+
31	(+)	(+)	–	+	–	–	+	(+)
33	–	–	+	–	–	–	–	–
34	–	–	–	–	–	nd	(+)	(+)
36	+	+	–	–	–	–	–	–
39	+	+	–	–	–	–	–	–
40	(+)	–	–	–	–	–	–	–

17, 46 are groups of proteins. + = well expressed; (+) = moderately expressed; – = absent or very weakly expressed; nd = not detectable or uncertain.

*For numbers 42–50, see Figure 7, for numbers 1–24, see Figure 3; for numbers 25–41, see Figure 5.

TABLE IV. Stage- and Lineage-Related Proteins During Early Postimplantation Development*

Tissues	Proteins	Day 7	Day 8	Day 9
Trophoblast, ectoplacental cone and placental anlage	4, 8, 11, 19, 21, 22, 39, 40	××××××××××××××××××××××→		
	25, 28, 36, 54		××××××××××××××→	
	15	××××		
	23, 26, 27, 32, 33, 37, 38		××××	
	42, 43, 49, 50			××××→
Extraembryonic tissues, amnion, allantois and yolk sac	4, 13, 47	××××××××××××××××××××××→		
	23, 30	××××××××××××××		
	33		××××××××××××××→	
	3, 14, 15	××××		
	32, 34		××××	
	46, 48			××××→
Embryonic tissues	5, 30, 47	××××××××××××××××××××××→		
	34, 45		××××××××××××××→	
	3, 13, 23	××××		
	32, 35		××××	
	44, 51, 52, 53			××××→

*Thirty-nine proteins or groups of proteins that persist, appear, or disappear during the developmental period analyzed.

At day 9, the yolk sac shows conspicuous changes in newly synthesized proteins toward a less complex pattern when compared with its previous one at day 8. In addition, a group of very acid proteins (Fig. 7C) in the range of about 60 kD appear. Protein synthesis in the allantois, amnion, and placental anlage is reduced and quite different from day 8, most likely as a result of functional changes. On the other hand, protein synthesis in the trophoblast remains almost the same, and only four new proteins could be detected. With respect to tissues of the embryo, we have not seen differences in newly synthesized proteins in the cranial and caudal part. For the heart, however, at least three new less acid proteins in the range of about 55 kD appear, and a fourth protein persists that is already seen only in the middle part of the day 8 embryo. Blood contamination, which is responsible for the appearance of these proteins, can be ruled out because these four spots are present neither in the yolk sac containing haematopoietic cells nor in other parts of the embryo.

Although it may be predictable that there is a difference in protein synthesis of the different tissues or developmental stages analyzed, we would like to emphasize that this nevertheless first must be discovered. After having documented that particular proteins can be found that are expressed in specific tissues, that persist in certain cell lineages, and therefore may be considered related to the processes of morphogenesis and differentiation, they can be utilized for identifying the corresponding genes. We are, of course, aware of the fact that currently we do not have any evidence for the functional role of these proteins. One possible approach toward this goal could be to isolate from the gels the protein spots of interest for amino acid sequencing and corresponding polypeptide synthesis used for cDNA synthesis. It also will be of interest to pursue our studies on tissue- and stage-specific protein synthesis beyond day 9 of postimplantation development and to reveal further tissue- and stage-specific gene expression during mammalian differentiation.

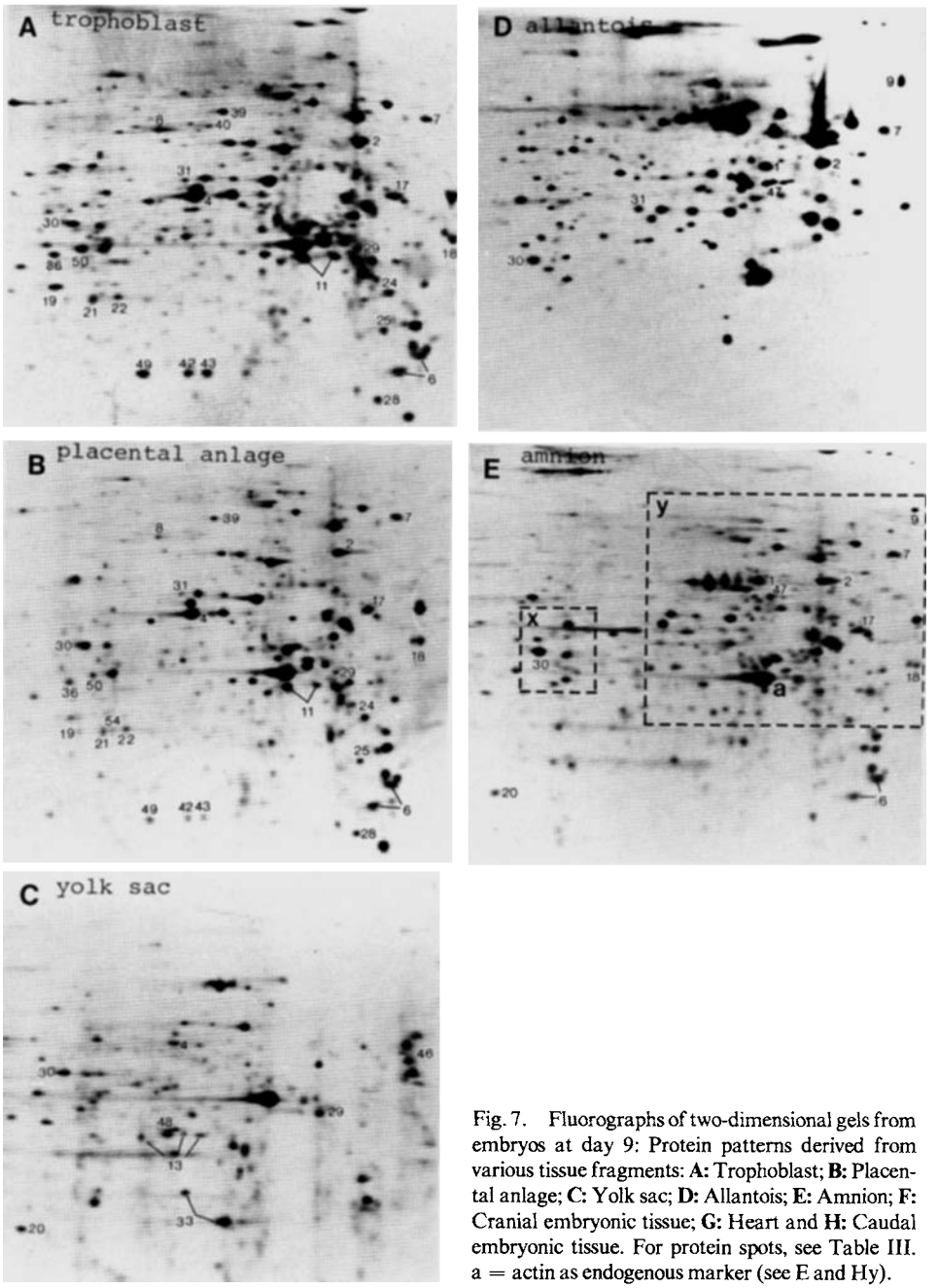

Fig. 7. Fluorographs of two-dimensional gels from embryos at day 9: Protein patterns derived from various tissue fragments: **A:** Trophoblast; **B:** Placental anlage; **C:** Yolk sac; **D:** Allantois; **E:** Amnion; **F:** Cranial embryonic tissue; **G:** Heart and **H:** Caudal embryonic tissue. For protein spots, see Table III. a = actin as endogenous marker (see E and Hy).

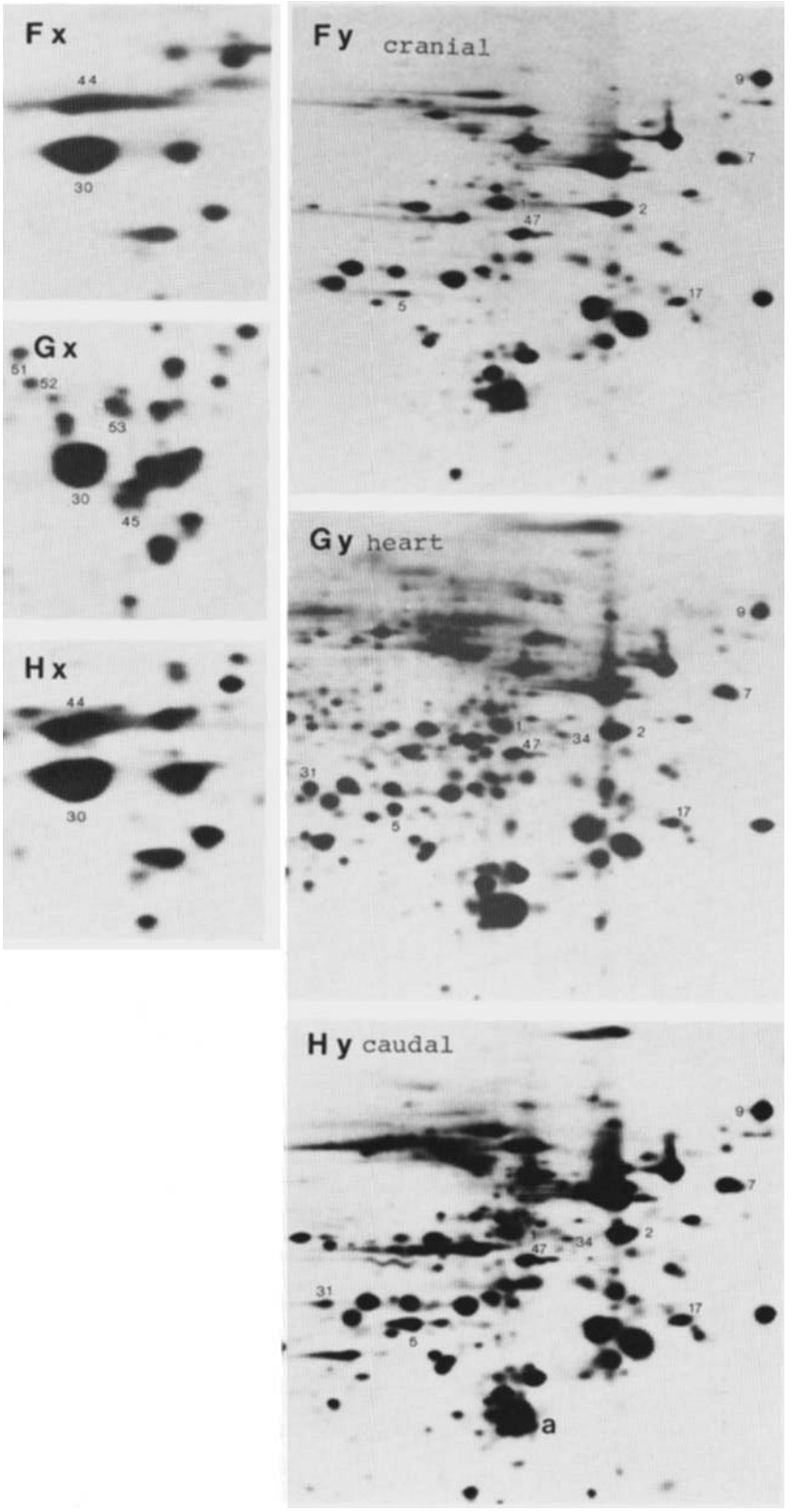
Fx
44
30
Fy cranial
9
7
2
47
5
17
Gx
51
52
53
30
45
Gy heart
9
7
1
34
2
47
31
5
17
Hx
44
30
Hy caudal
9
7
2
34
47
31
5
17
a

Figure 7F–G

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