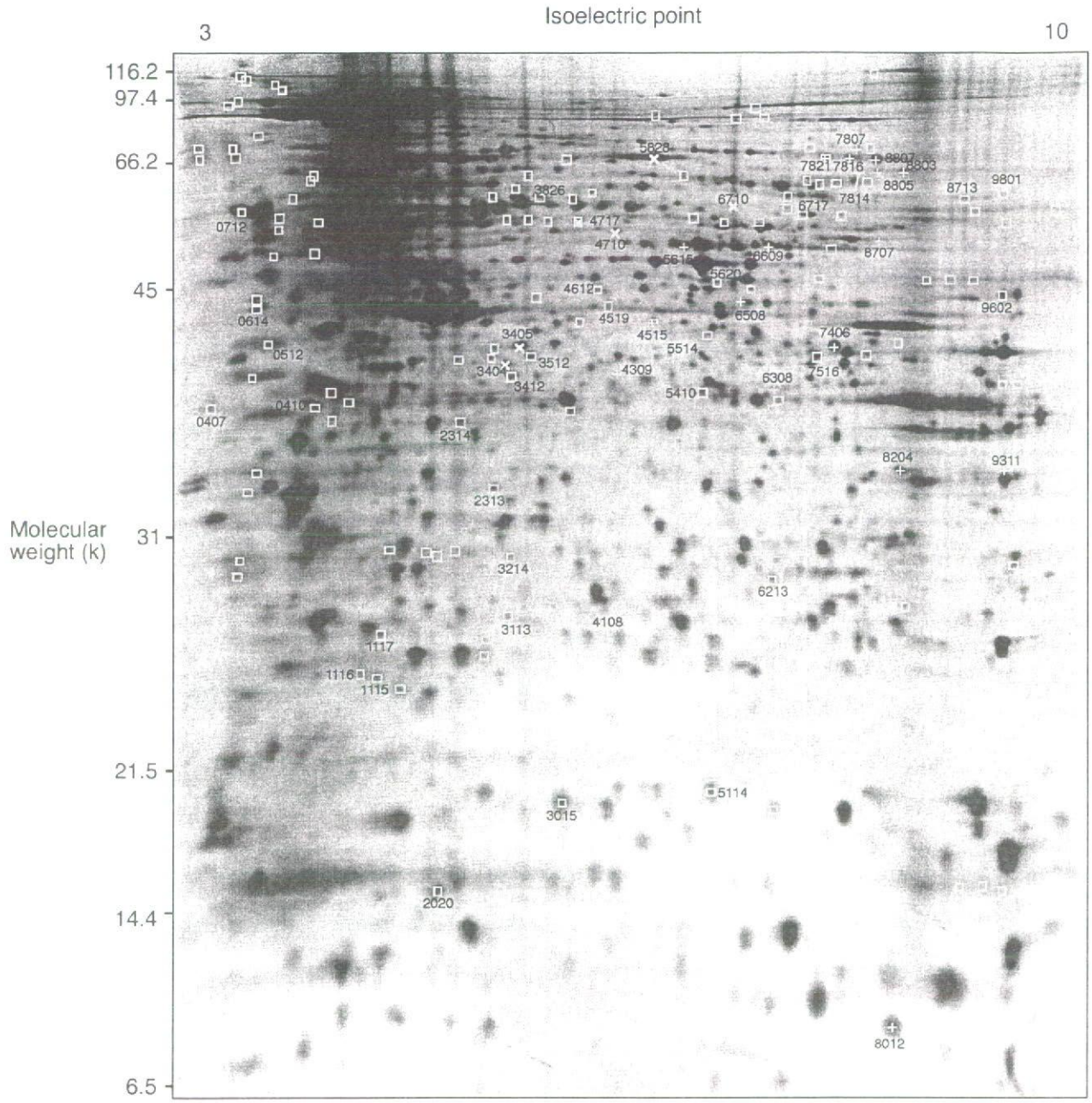

Fig. 2. 2-DE gel of the embryo proper from a cultured rat embryo equivalent to day 11.5. Quantitatively different spots between the 48 and 24 h culture are indicated by a cross (larger in 48 h) or a plus (larger in 24 h) with their SSP. Embryo proper specific spots are indicated by a square and their SSP is shown if its protein was identified.

proteins that were thought to play important roles in developmental process, for example, acidic calponin (SSP 3512), cellular retinoic acid binding protein 2 (SSP 2020), cofilin 1 (SSP 5114), myosin light chain 1 (SSP 1117), and stathmin 1 (SSP 3015) in the embryo proper, and Ash-m (SSP 4214), dimerization cofactor of hepatocyte nuclear factor 1 alpha (SSP 5005), ERM-binding phosphoprotein (SSPs 4303, 4701), prepro-cathepsin L (SSP 0302), and legumain precursor (SSPs 3515, 4503) in the yolk sac membrane. It was noted that multiple spots of complement component 3 (SSPs 1010, 1017, 2108, 2311, 3006, 4105, 4304, 4306, 5111, 8009), which appeared smaller than its intact molecule, were detected only in the yolk sac but not in the embryo proper.

Some proteins were detected in both embryo proper and yolk sac membranes as different protein spots. Adenosine kinase, eukaryotic translation initiation factor 4E, nucleophosmin 1, and protein SET were detected in both embryo proper (SSPs 4612, 3214, 0410, 0512, 0614) and yolk sac membrane (SSPs 4505, 4109, 0004, 4104), but their spots were different (Tables 4–6). Malate dehydrogenase was detected as a soluble form in the embryo

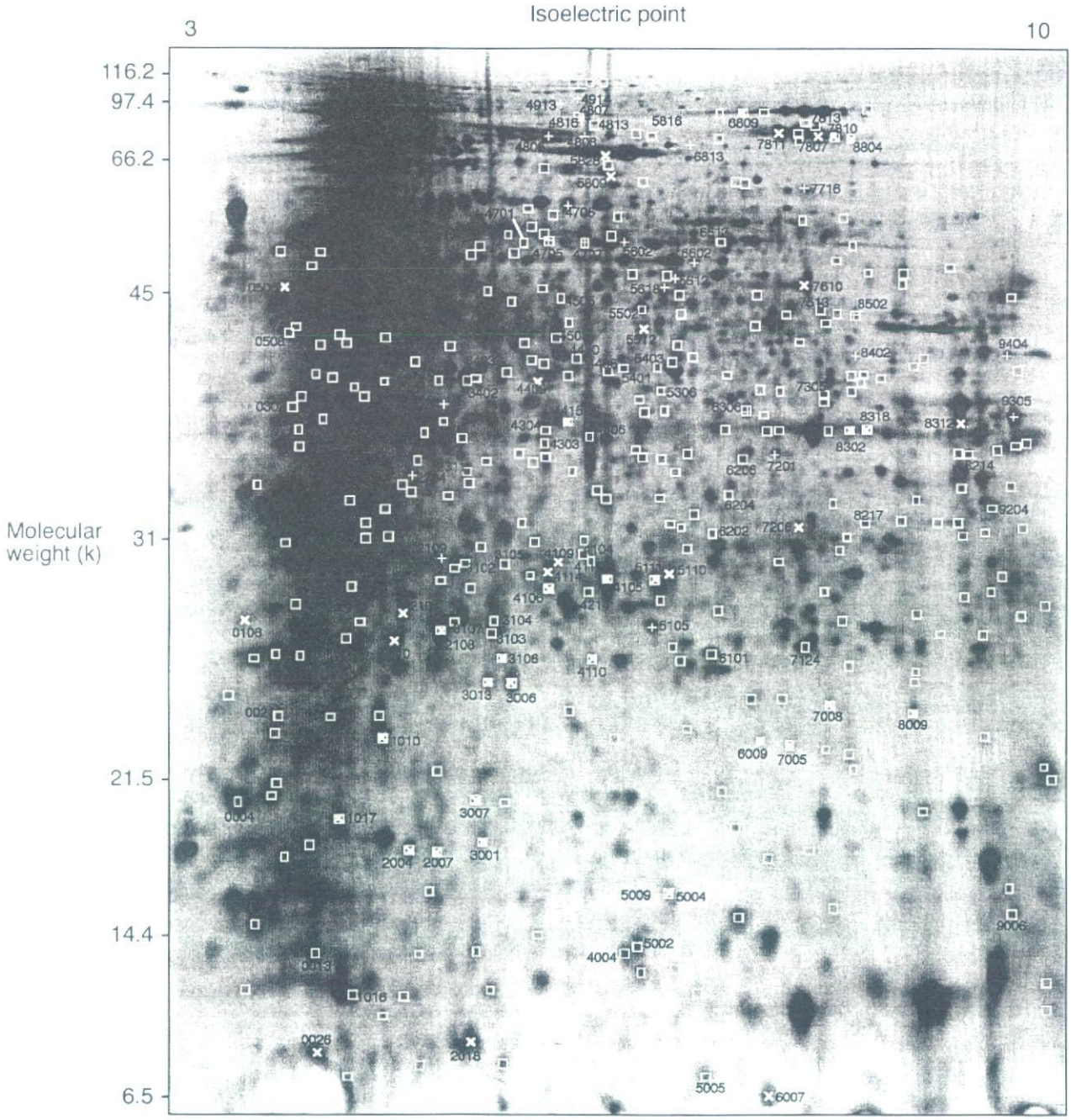

Fig. 3. 2-DE gel of the yolk sac membrane from a cultured rat embryo equivalent to day 11.5. Quantitatively different protein spots between the 48 and 24 h culture are indicated by a cross (larger in 48 h) or a plus (larger in 24 h) with their SSP. Yolk sac membrane specific spots are indicated by a square and their SSP is shown if its protein was identified.

proper (SSP 2314) and as a mitochondrial form in the yolk sac membrane (SSP 8214).

DISCUSSION

The present study showed 2-DE profiles of cultured rat embryos at an early postimplantation stage. When 2-DE profiles of the embryo proper and yolk sac membrane were compared between the 48 and 24 h cultures, there were quantitative but no qualitative differences in the protein spots. It has been shown that specific proteins were observed corresponding to the developmental stages in the postimplantation rodent embryos when analyzed by 2-DE (Greene et al., 2002). It is, therefore, considered that the developmental stage reached by the cultured rat embryos is comparable irrespective of the length of culture period in spite of varied growth as determined by the size of embryos.

Quantitative differences in the protein spots between the 48 and 24 h culture might be related to the varied embryonic growth. It is considered that larger-quantity proteins including RNA-binding proteins in the embryo proper might support better embryonic growth in the 24 h culture. On the other hand, the yolk sac membrane in the 48 h culture seems to have stored an excess amount of serum proteins from the culture medium that should have been digested and supplied as nutrients (Lloyd et al., 1998) to the embryo proper. In the yolk sac membrane, a smaller quantity of constitutive enzymes, relative to the larger quantity in the 24 h culture, may indicate less activity of the yolk sac membrane in the 48 h culture.

Table 2
Summary of Quantitative Differences in 2-DE Profiles of Rat Embryos at day 11.5 by the Varied Length of Culture Period

	Total number of matched spots	Larger quantity in 48 h culture from day 9.5	Larger quantity in 24 h culture from day 10.5
Embryo proper	853	7	18
Yolk sac membrane	1,087	45	26

The number of protein spots whose quantities are significantly different between the 48 and 24 h culture by the *t*-test ($P < 0.01$) is shown. The distribution of the number of larger-quantity spots between the embryo proper and yolk sac membrane is significantly different when analyzed by the Fisher's exact test ($P = 0.0015$).

From the protein spots specific to either of the embryo proper or yolk sac membrane, proteins involved in tissue-characteristic functions, such as morphogenesis in the former and nutritional transfer in the latter, could be identified. In the embryo proper, acidic calponin may be involved in brain development (Ferhat et al., 1996). Cellular retinoic acid binding protein 2 has some role in retinoic acid synthesis and retinoid signaling during morphogenesis (Ruberte et al., 1992; Zheng et al., 1999). Cofilin 1 is developmentally regulated, and is required for neural tube morphogenesis and neural crest cell migration (Greene et al., 2002; Gurniak et al., 2005). Myosin light chain 1 is expressed in cardiac development (Lyons et al., 1990). Stathmin 1 may regulate cell proliferation, differentiation, and maturation (Koppel et al., 1990). In the yolk sac membrane, Ash-m may regulate endocytosis for the uptake of nutrients (Miki et al., 1994; Watanabe et al., 1995). Dimerization cofactor of hepatocyte nuclear factor 1 alpha may regulate yolk sac–specific transthyretin expression (Cereghini et al., 1992; Costa et al., 1990). ERM-binding phosphoprotein might be involved in receptor recycling in endocytosis (Brent et al., 1990; Li et al., 2002). Cathepsin L (Sol-Church et al., 1999) and legumain (Ambroso and Harris, 1994; Shirahama-Noda et al., 2003) are lysosomal proteases important for histiotrophic nutrition.

Multiple spots of complement component 3 (C3) in the yolk sac membrane are considered to be the digestive

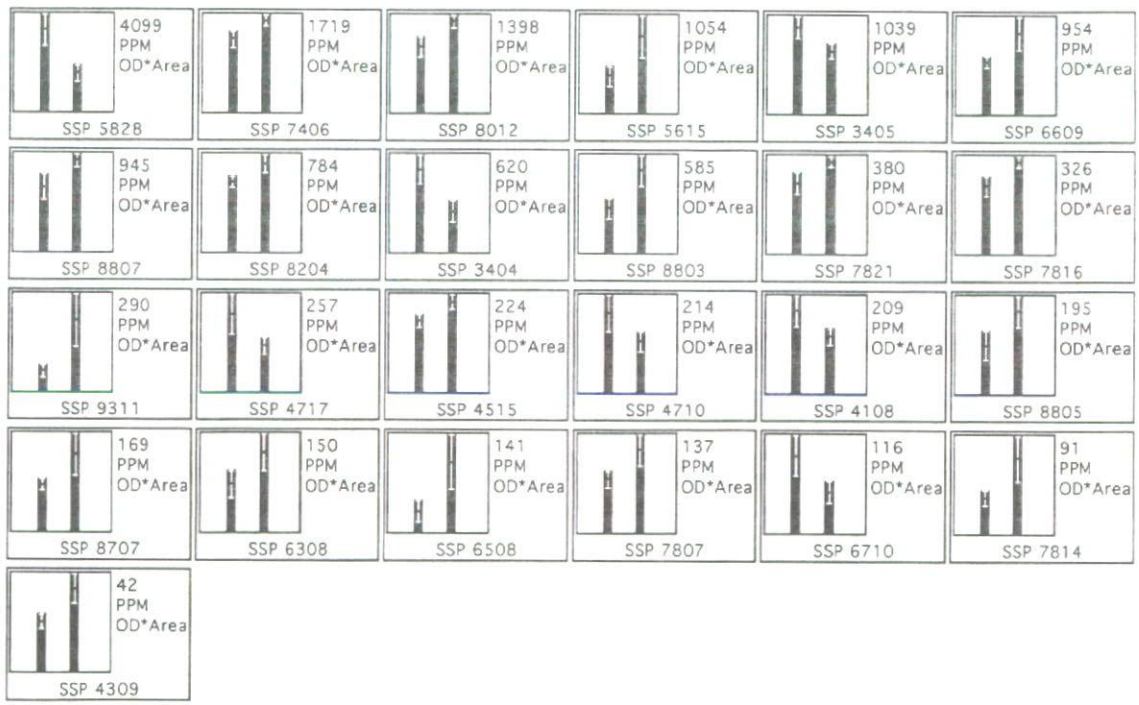

Fig. 4. Quantity of protein spots with significant differences between the 48 and 24 h culture in the embryo proper. Mean and SD ranges are shown in the bars (left for 48 h and right for 24 h) in the boxes for individual protein spots as indicated by SSP. The boxes are arranged from the top left to the right in the descending order of the spot quantity in the 48 h culture. Mean quantity of the larger-quantity spots between the 48 and 24 h culture are indicated as ppm of the total density in the gel image at the top right in each box.

Fig. 5. Quantity of protein spots with significant differences between the 48 and 24 h culture in the yolk sac membrane. Mean and SD ranges are shown in the bars (left for 48 h and right for 24 h) in the boxes for individual protein spots as indicated by SSP. The boxes are arranged from the top left to the right in the descending order of the spot quantity in the 48 h culture. Mean quantity of the larger-quantity spots between the 48 and 24 h culture are indicated as ppm of the total density in the gel image at the top right in each box.

Table 3
Identified Protein Spots With Quantitative Differences Between the 24 and 48 h Culture in the Embryo Proper

SSP	Protein name	Nominal mass	Calculated pI	Accession No.
Larger quantity in 48 h culture				
4108	DJ-1 protein [Rattus norvegicus]	20190	6.32	gi|16924002
5828	Albumin [Rattus norvegicus]	70670	6.09	gi|19705431
Larger quantity in 24 h culture				
4515	Poly(rC) binding protein 2 [Rattus norvegicus]	38955	6.33	gi|61557337
	AND			
	TAR DNA binding protein isoform 1 [Mus musculus]	44918	6.36	gi|21704096
5615	Mitochondrial aldehyde dehydrogenase precursor [Rattus norvegicus]	56079	6.69	gi|45737866
7406	PREDICTED: similar to Poly(rC)-binding protein 1 (Alpha-CP1)(hnRNP-E1) [Rattus norvegicus]	37987	6.66	gi|6754994
7807	Transferrin [Rattus norvegicus]	78512	7.14	gi|61556986
7821	Far upstream element (FUSE) binding protein 1 [Rattus norvegicus]	67326	7.18	gi|83320094
8012	Macrophage migration inhibitory factor	12709	7.74	gi|694108
8204	B-36 VDAC = 36 kda voltage dependent anion channel [rats, hippocampus, Peptide, 295 aa]	32327	7.44	gi|299036
8803	Transketolase [Rattus norvegicus]	71940	7.54	gi|12018252
8807	Far upstream element (FUSE) binding protein 1 [Rattus norvegicus]	67326	7.18	gi|83320094

Table 4
Identified Protein Spots With Quantitative Differences Between the 24 and 48 h Culture in the Yolk Sac Membrane

SSP	Protein name	Nominal mass	Calculated pI	Accession No.
Larger quantity in 48 h culture				
0026	Tubulin, alpha 1 [Rattus norvegicus]	50816	4.94	gi|38328248
	OR			
	Tubulin, alpha 6 [Rattus norvegicus]	50590	4.96	gi|58865558
0106	Membrane-associated progesterone receptor component 1 (Acidic 25-kDa protein) (25-DX)	21699	4.45	gi|6647578
0506	Reticulocalbin 3, EF-hand calcium binding domain [Rattus norvegicus]	37918	4.72	gi|56744249
1010	PREDICTED: similar to Programmed cell death protein 6 (Probable calcium-binding protein ALG-2) (PMP41) (ALG-257) [Rattus norvegicus]	21948	5.36	gi|34853323
	AND			
	Alpha-1-macroglobulin	168388	6.46	gi|202857
	OR			
	Pregnancy-zone protein [Rattus norvegicus]	168422	6.46	gi|21955142
	AND			
	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
1017	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
2018	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
2108	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
3001	Actin related protein 2/3 complex, subunit 5 [Rattus norvegicus]	16367	5.47	gi|71043638
3006	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
4105	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
4109	Eukaryotic translation initiation factor 4E [Rattus norvegicus]	25266	5.79	gi|16758870
4415	Serine (or cysteine) peptidase inhibitor, clade F, member 2 [Rattus norvegicus]	55086	5.74	gi|58865362
5111	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
	AND			
	Heat shock 90-kDa protein 1, beta [Rattus norvegicus]	83631	4.97	gi|51859516
5828	Albumin [Rattus norvegicus]	70670	6.09	gi|19705431
7807	Transferrin [Rattus norvegicus]	78512	7.14	gi|61556986
8009	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024

Table 4
Continued

SSP	Protein name	Nominal mass	Calculated pI	Accession No.
8302	Glyceraldehyde-3-phosphate dehydrogenase [Rattus norvegicus]	36090	8.14	gi|8393418
	AND			
	Aldolase A	39691	8.31	gi|202837
Larger quantity in 24 h culture				
4706	Protein disulfide-isomerase A3 precursor (Disulfide isomerase ER-60) (ERp60) (58 kDa microsomal protein) (p58) (ERp57) (HIP-70) (Q-2)	57044	5.88	gi|1352384
4707	Liver annexin-like protein [Rattus norvegicus]	52036	5.99	gi|7108713
	OR			
	Plasma glutamate carboxypeptidase [Rattus norvegicus]	51994	6.09	gi|13928880
	OR			
	Pgcp protein [Rattus norvegicus]	52066	5.99	gi|47477878
5602	Liver annexin-like protein [Rattus norvegicus]	52036	5.99	gi|7108713
5618	PREDICTED: similar to adenylosuccinate synthetase, non muscle [Rattus norvegicus]	50453	5.98	gi|34880967
6602	Enolase 1, alpha [Rattus norvegicus]	47428	6.16	gi|6978809
7201	PREDICTED: similar to Glucosamine-6-phosphate isomerase (Glucosamine-6-phosphate deaminase) (GNPDA) (GlcN6P deaminase) (Oscillin) [Rattus norvegicus]	32730	6.27	gi|109507234
8502	Arginosuccinate synthetase [Rattus norvegicus]	46752	7.63	gi|25453414
9404	Peroxisomal 3-ketoacyl-CoA thiolase A [Rattus norvegicus]	45324	8.53	gi|220849

Table 5
Identified Protein Spots Specific to the Embryo Proper of Cultured Rat Embryos

SSP	Protein name	Nominal mass	Calculated pI	Accession No.
0407	Methylosome subunit pICln (Chloride conductance regulatory protein ICln) (I(Cln)) (Chloride channel, nucleotide sensitive 1A)	26190	3.97	gi|544031
0410	Nucleophosmin 1 [Rattus norvegicus]	32711	4.62	gi|7242160
0512	Protein SET (Phosphatase 2A inhibitor I2PP2A) (I-2PP2A) (Template-activating factor I) (TAF-I) (Liver regeneration-related protein LRRGR00002)	33386	4.22	gi|46396568
0614	Protein SET (Phosphatase 2A inhibitor I2PP2A) (I-2PP2A) (Template-activating factor I) (TAF-I) (Liver regeneration-related protein LRRGR00002)	33386	4.22	gi|46396568
0712	Nucleosome assembly protein 1-like 1 [Rattus norvegicus]	45630	4.36	gi|77404363
1115	PREDICTED: similar to Chromobox protein homolog 5 (Heterochromatin protein 1 homolog alpha) (HP1 alpha) [Rattus norvegicus].	22343	5.71	gi|27665448
1116	Chromobox homolog 3 [Rattus norvegicus].	20969	5.23	gi|15082258
1117	Myosin light polypeptide 4 (Myosin light chain 1, atrial isoform)	21383	4.96	gi|127140
2020	Cellular retinoic acid binding protein 2 [Rattus norvegicus]	16094	5.23	gi|25453404
2313	p32-Subunit of replication protein A [Rattus norvegicus]	28980	5.47	gi|1403534
2314	Malate dehydrogenase 1, NAD (soluble) [Rattus norvegicus]	36631	6.16	gi|15100179
	OR			
	Lactate dehydrogenase A [Rattus norvegicus]	36712	8.45	gi|8393706
	AND			
	Dermal papilla derived protein 6 [Rattus norvegicus]	35810	5.15	gi|48843729
3015	Stathmin 1 [Rattus norvegicus]	17278	5.76	gi|8393696
3113	RAB11B, member RAS oncogene family [Rattus norvegicus]	24588	5.64	gi|14249144
3214	Eukaryotic translation initiation factor 4E [Rattus norvegicus]	25266	5.79	gi|16758870
3412	PREDICTED: similar to Eukaryotic translation initiation factor 3 subunit 2 (eIF-3 beta) (eIF3 p36) (eIF3i) (TGF-beta receptor-interacting protein 1) (TRIP-1) [Rattus norvegicus]	36837	5.38	gi|109477318
3512	Calponin 3, acidic [Rattus norvegicus]	36583	5.47	gi|9506501
3826	Serine/threonine-protein kinase PAK 2 (p21-activated kinase 2) (PAK-2) (Gamma-PAK) (p62-PAK)	58209	5.57	gi|2499648
	AND			
	Myo-inositol 1-phosphate synthase A1 [Rattus norvegicus]	48551	5.67	gi|62078495

Table 5
Continued

SSP	Protein name	Nominal mass	Calculated pI	Accession No.
4515	Poly(rC) binding protein 2 [Rattus norvegicus]	38955	6.33	gi|61557337
	AND			
	TAR DNA binding protein isoform 1 [Mus musculus]	44918	6.36	gi|21704096
4519	PREDICTED: similar to protein phosphatase methylesterase 1 [Rattus norvegicus]	42688	5.67	gi|62641013
4612	Adenosine kinase [Rattus norvegicus]	40415	5.84	gi|1906013
	AND			
	2-Oxoisovalerate dehydrogenase subunit alpha, mitochondrial precursor (Branched-chain alpha-keto acid dehydrogenase E1 component alpha chain) (BCKDH E1-alpha)	50418	7.68	gi|129032
5114	Cofilin 1 [Rattus norvegicus]	18749	8.22	gi|8393101
5410	Protein phosphatase 1, catalytic subunit, gamma isoform [Rattus norvegicus]	37701	6.12	gi|11968062
	AND			
	mRNA decapping enzyme [Rattus norvegicus]	38804	6.02	gi|23463287
	AND			
	PRP4 protein kinase homolog [Mus musculus]	96187	10.45	gi|3236351
5514	PREDICTED: similar to RIKEN cDNA 1110064P04 [Rattus norvegicus]	45347	7.63	gi|109500109
5620	PREDICTED: similar to DEAD (Asp-Glu-Ala-Asp) box polypeptide 48 [Rattus norvegicus]	47096	6.3	gi|109492318
	AND			
	C-Terminal binding protein 1 [Rattus norvegicus]	47055	6.17	gi|15011859
6213	Hypothetical protein LOC302247 [Rattus norvegicus]	33918	8.73	gi|114145722
6717	Nucleoporin 54kDa [Rattus norvegicus]	55825	6.53	gi|8393855
7516	Arginase-1 (Type I arginase) (Liver-type arginase)	35122	6.76	gi|114146
	AND			
	PREDICTED: similar to peptidylprolyl isomerase D [Rattus norvegicus]	41084	6.48	gi|62655561
7821	Far upstream element (FUSE) binding protein 1 [Rattus norvegicus]	67326	7.18	gi|83320094
8713	Far upstream element (FUSE) binding protein 3 [Rattus norvegicus]	61657	8.45	gi|86129564
9602	Serine (or cysteine) proteinase inhibitor, clade H, member 1 [Rattus norvegicus]	46602	8.88	gi|8393057
9801	Far upstream element (FUSE) binding protein 3 [Rattus norvegicus]	61657	8.45	gi|86129564

fragments from C3 in the culture medium since rat serum used as a culture medium contains a high concentration of C3 (>2 mg/ml) and C3 production in the yolk sac membrane is not known (Colten, 1994; Guiguet et al., 1987). Possibly, each of the multiple spots might have different biological functions because it is known that fragments of C3 function as various effectors. For example, C3b fragment enhances phagocytic activity in mouse placental trophoblasts probably through the CR1 receptor (Albieri et al., 1999; Amarante-Paffaro et al., 2004), and iC3b fragment functions as an embryotrophic factor probably through the Crry and CR3 receptor in mouse blastocysts (Lee et al., 2004).

Different spots of the same proteins between the embryo proper and yolk sac membrane may indicate the presence of isoforms or posttranslational modification of the proteins. There are at least two isoforms of adenosine kinase with tissue-dependent expression in rats (Sakowicz et al., 2001). Eukaryotic translation initiation factor 4E is phosphorylated and cleaved upon apoptosis (Scheper and Proud, 2002). The nucleophosmin spot in the yolk sac membrane may be due to proteolytic degradation that can be influenced by exogenous stimulation (Zhang et al., 2006). Three protein SET spots (two in the embryo proper and one in the yolk sac membrane) could be assigned to three kinds of transcript although their differential expression is not known (Fukukawa et al., 2000). Both soluble and mitochondrial forms of malate dehydrogenase are developmentally regulated and show tissue-specific expression (Greene et al., 2002; Kelly et al., 1989; Lo et al., 2005).

The protein identification data in the present study is consistent with those in our previous study (Usami et al., 2007) and added many identified spots to the 2-DE map of cultured postimplantation rat embryos. Protein spots analyzed in common in the embryo proper between these studies were identified as the same proteins, respectively, verifying the reproducibility of this method; for example, albumin (SSP 5828 and Spot No. 2 in the previous study), hnRNP-E1 (SSP 7406 and Spot No. 81), and stathmin 1 (SSP 3015 and Spot No. 29). From the updated 2-DE map, cofilin 1 (SSP 5114) is considered to be a charge variant probably due to phosphorylation because of its acidic pI sifted from that of previously identified cofilin 1 (Spot No. 25) (Usami et al., 2007) and known functional regulation of cofilin 1 (Sumi et al., 2001). Thus, the 2-DE map is useful across proteomic studies on cultured postimplantation rat embryos.

In conclusion, comparative proteome analysis of the embryo proper and yolk sac membrane of day 11.5 cultured rat embryos by 2-DE showed protein spots specific to each of them that can be related their

Table 6
Identified Protein Spots Specific to the Yolk Sac Membrane of Cultured Rat Embryos

SSP	Protein name	Nominal mass	Calculated pI	Accession No
0004	Nucleophosmin 1 [Rattus norvegicus]	32711	4.62	gi|38014709
0013	PREDICTED: hypothetical protein XP_579384 [Rattus norvegicus]	187746	6.06	gi|62718645
0021	Nucleolin [Rattus norvegicus]	77158	4.67	gi|6981248
0302	PREDICTED: tumor rejection antigen gp96 (predicted) [Rattus norvegicus]	92998	4.72	gi|62651904
	AND			
	Prepro-cathepsin L [Rattus norvegicus]	38232	6.73	gi|55888
0508	Heat shock 90kDa protein 1, beta [Rattus norvegicus]	83631	4.97	gi|51859516
1010	PREDICTED: similar to programmed cell death protein 6 (Probable calcium-binding protein ALG-2) (PMP41) (ALG-257) [Rattus norvegicus]	21948	5.36	gi|34853323
	AND			
	Alpha-1-macroglobulin	168388	6.46	gi|202857
	OR			
	Pregnancy-zone protein [Rattus norvegicus]	168422	6.46	gi|21955142
	AND			
	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
1016	PREDICTED: similar to germinal histone H4 gene [Rattus norvegicus]	18394	10.93	gi|62648434
	OR			
	PREDICTED: similar to Histone H2B 291B [Rattus norvegicus]	37149	10.56	gi|62663604
1017	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
1101	Chain A, Catechol O-Methyltransferase	24960	5.11	gi|1633081
	AND			
	Proteasome endopeptidase complex (EC 3.4.25.1) delta chain - rat	21864	4.98	gi|285140
2108	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
2311	3-Hydroxyanthranilate 3,4-dioxygenase [Rattus norvegicus]	32846	5.57	gi|9910256
	AND			
	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
3001	Actin related protein 2/3 complex, subunit 5 [Rattus norvegicus]	16367	5.47	gi|71043638
3006	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
3102	Phosphoserine phosphatase [Rattus norvegicus]	25180	5.49	gi|57527332
3103	Apolipoprotein A-I [Rattus norvegicus]	30100	5.52	gi|6978515
3104	Apolipoprotein A-I [Rattus norvegicus]	30100	5.52	gi|6978515
3105	Phosphoserine phosphatase [Rattus norvegicus]	25180	5.49	gi|57527332
3107	Apolipoprotein A-I [Rattus norvegicus]	30100	5.52	gi|6978515
3402	PREDICTED: similar to Tubulin alpha-2 chain (Alpha-tubulin 2) [Rattus norvegicus]	50932	4.94	gi|62653035
	AND			
	Actin beta - rat	42066	5.29	gi|71620
3403	Eukaryotic translation initiation factor 3, subunit 2 (beta) [Mus musculus]	36837	5.38	gi|9055370
3515	Legumain precursor (Asparaginyl endopeptidase) (Protease, cysteine 1)	49834	6.09	gi|22001735
4004	Expressed in non-metastatic cells 2 [Rattus norvegicus]	17386	6.92	gi|55926145
4104	Protein SET (Phosphatase 2A inhibitor I2PP2A) (I-2PP2A) (Template-activating factor I) (TAF-I) (Liver regeneration-related protein LRRGR00002)	33386	4.22	gi|46396568
4105	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
4115	Chaperonin subunit 6a (zeta) [Rattus norvegicus]	58437	6.63	gi|76253725
4214	Ash-m [Rattus norvegicus]	23655	6.31	gi|914957
4303	ERM-binding phosphoprotein [Rattus norvegicus]	39149	5.7	gi|11024674
4304	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
4306	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
4408	Eukaryotic translation elongation factor 2 [Rattus norvegicus]	96192	6.41	gi|8393296
4410	Elongation factor 2	38690	5.69	gi|203997
4415	Serine (or cysteine) peptidase inhibitor, clade F, member 2 [Rattus norvegicus]	55086	5.74	gi|58865362
4503	Legumain precursor (Asparaginyl endopeptidase) (Protease, cysteine 1)	49834	6.09	gi|22001735
4505	Adenosine kinase [Rattus norvegicus]	40415	5.84	gi|1906013
4701	ERM-binding phosphoprotein [Rattus norvegicus]	39149	5.7	gi|11024674

Table 6
Continued

SSP	Protein name	Nominal mass	Calculated pI	Accession No
4707	Liver annexin-like protein [Rattus norvegicus] OR	52036	5.99	gi|7108713
	Plasma glutamate carboxypeptidase [Rattus norvegicus] OR	51994	6.09	gi|13928880
	Pgcp protein [Rattus norvegicus]	52066	5.99	gi|47477878
4813	Fatty acid synthase	275117	5.96	gi|204099
5002	Transthyretin precursor (Prealbumin) (TBPA)	15824	5.77	gi|136467
5005	Chain A, crystal structure of a complex between the dimerization domain of Hnf-1 alpha and the coactivator Dcoh	11989	6.06	gi|10835702
5111	Complement component 3 [Rattus norvegicus] AND	187825	6.12	gi|8393024
	Heat shock 90-kDa protein 1, beta [Rattus norvegicus]	83631	4.97	gi|51859516
5306	M2 pyruvate kinase [Rattus norvegicus]	58314	7.15	gi|206205
5401	Elongation factor 2	38690	5.69	gi|203997
5403	Gale protein [Rattus norvegicus]	38671	6.02	gi|67678395
5502	Enolase 1, alpha [Rattus norvegicus]	47428	6.16	gi|6978809
6101	Chain A, Crystal Structure Of A Mammalian 2-Cys Peroxiredoxin, Hbp23.	22250	8.34	gi|6435547
6202	Similar to RIKEN cDNA 2810409H07 [Rattus norvegicus]	44792	7.62	gi|76677911
6204	Eukaryotic translation initiation factor 4A, isoform 1 [Rattus norvegicus]	46353	5.32	gi|40786436
6206	Elongation factor 2	38690	5.69	gi|203997
6613	PREDICTED: similar to aldehyde dehydrogenase family 7, member A1 [Rattus norvegicus]	59225	7.99	gi|62664437
7124	H(+)-transporting ATP synthase [Rattus norvegicus] AND	25639	7.03	gi|57029
	Chain A, crystal structure of a mammalian 2-Cys peroxiredoxin, Hbp23.	22250	8.34	gi|6435547
7305	Citrate synthase [Rattus norvegicus]	52176	8.53	gi|18543177
7513	Eukaryotic translation elongation factor 2 [Rattus norvegicus]	96192	6.43	gi|8393296
7813	Serotransferrin precursor (Transferrin) (Siderophilin) (Beta-1-metal-binding globulin) OR	78538	6.94	gi|6175089
	Ab2-417 [Rattus norvegicus]	109510	8.35	gi|33086606
8009	Complement component 3 [Rattus norvegicus]	187825	6.12	gi|8393024
8214	Malate dehydrogenase, mitochondrial [Rattus norvegicus]	36117	8.93	gi|42476181
8217	Adenylate kinase 2 isoform a [Rattus norvegicus] OR	26648	6.33	gi|13591872
	Adenylate kinase 2 isoform b [Rattus norvegicus]	25741	7.01	gi|77020256
8302	Glyceraldehyde-3-phosphate dehydrogenase [Rattus norvegicus] AND	36090	8.14	gi|8393418
	Aldolase A	39691	8.31	gi|202837
8502	Arginosuccinate synthetase [Rattus norvegicus]	46752	7.63	gi|25453414
9006	Eukaryotic translation elongation factor 1 alpha 1 [Rattus norvegicus]	50424	9.1	gi|28460696
9204	Transaminase, Glu oxaloacetic	44678	8.52	gi|350611

characteristic functions. 2-DE profiles of cultured postimplantation rat embryos are considered quantitatively variable according to the culture conditions but qualitatively comparable. Proteomic analysis of cultured postimplantation rat embryos will be a new approach in developmental biology and toxicology at the protein level.

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