line (Fig. 1A, 1B). The annotations for 28 differentially expressed features are listed in Table 1. Of the 28 differential features identified, we further selected proteins whose mean abundance values differed by ≥ 2 fold. In total, 24 proteins met this criterion, and their expression profiles in the course of 72 h after X-irradiation are depicted in Fig. 2.

Eleven proteins exhibited significant abundance alterations in late time intervals from 24 to 48 h, in which the formation of MN is started and reaches its maximum. This group of spots involved rho GDP-dissociation inhibitor 2 (RhoGDI 2), two variants of RIK protein, epsilon subunit of T-complex protein 1 (TCP-1), regulatory subunit of proteasome complex, enzyme transketolase, metal response element-binding transcription factor (MTF2), translationally controlled tumour protein (TCTP), histidine triad nucleotide-binding protein (HINT), aldose reductase (ALDR) and nucleoside diphosphate kinase B (NDK B). The expression of seven proteins (protein disulphide isomerase (PDI) A6 precursor, Rab 1, 14-3-3 protein zeta/delta, pyruvate kinase, initiation factor 5A (eIF 5A), rho GDP-dissociation inhibitor 1 (RhoGDI 1) and L04851 putative protein) was altered in both early and late examined periods. Epididymal 17 kDa precursor was then an example of early radiation-induced abundance alteration and, conversely, the level of five proteins (2610511I09RIK protein, peroxiredoxin 4, vascular endothelial growth factor D (VEGF-D), inosin-5'-monophosphate dehydrogenase 2 (IMPDH-II) and 40S ribosomal protein) was altered in the last examined interval 72 h. Partial 2-DE images showing differences in the protein expression level of spots exhibiting significant 2-fold abundance alterations after irradiation are denoted in Fig. 3A and 3B.

Discussion

Identification of new molecular biomarkers for radiation exposure assessment can have profound beneficial applicability in radiation epidemiology, therapy and biodosimetry. Our strategy is to identify radiation-responsive gene expression targets using a comparative proteomics procedure. The main goal of this approach is the determination of qualitative and quantitative differences in the protein composition of two or more samples followed by identification of selected proteins (Smolka et al., 2002). Using an *in vitro* model system of murine L929 fibroblasts we searched for differentially expressed proteins in the course of three days after X-irradiation. We identified 24 proteins whose abundances were significantly altered by radiation. The identified proteins can be divided into seven functional groups referring to which cellular systems were targeted by ionizing radiation.

The first group involves five proteins engaged in cell signalling pathways. RhoGDI 1 and 2 are cytosolic proteins that participate in the regulation of both the GDP/GTP cycle and the membrane association/dissociation cycle of Rho/Rac proteins (Olofsson et al., 1999). Radiation regulates, especially in late intervals, protein levels of both RhoGDIs differentially. So, while the RhoGDI 2 level exhibits severe suppression from 24 to 48 h after irradiation, the amount of RhoGDI 1 exerts strong induction two days after treatment. The opposite regulation of RhoGDI 1 and RhoGDI 2 abundances was also described for drug-induced apoptosis (Essman et al., 2000). Rab 1 is a member of the Ras family of GTPases and it controls transport events through early Golgi compartments (Martinez and Goud, 1998). Rab 1 is exceptional among identified proteins because its level is besides 24 h upregulated by radiation in all examined time intervals. There is no information about the particular role of Rab 1 in irradiated cells. Nevertheless, there is substantial evidence to demonstrate that increased radiation resistance is accompanied with increased expression of other members of the Ras protein family (Jones et al., 2001). A further identified protein included in this group, the 14-3-3 zeta/delta, is probably a multifunctional regulator of the cell signalling processes mediated by both protein kinase II and protein kinase C. The 14-3-3 family members are dimeric phosphoserine-binding proteins, and primary function of mammalian 14-3-3 proteins is to inhibit apoptosis (Xing et al., 2000). Other authors (Hermeking et al., 1997) discovered that 14-3-3 sigma protein levels are strongly induced by gamma irradiation and other DNA-damaging agents in a colorectal cancer cell line. Our data correspond with the published finding and the increased level of 14-3-3 zeta/delta protein can substantiate the reluctance of L929 cells to activate the apoptotic process. HINT is an intracellular receptor for purine mononucleotides and it is an inhibitor of protein kinase C. Recently, 2-fold downregulation of HINT was observed in a human bronchial epithelial cell line irradiated by low doses of gamma-rays (Gamble et al., 2000). In our study, a significant decrease of HINT concentration was found 24 h after irradiation of L929 cells.

The second group of the identified proteins comprises two enzymes of carbohydrate metabolism, transketolase and pyruvate kinase, M2 isozyme. While transketolase was significantly upregulated in X-irradiated cells, the level of pyruvate kinase, M2 isozyme shows a more complicated pattern of expression, with two peaks at 12 and 36 h followed by the decline in the last two examined intervals 48 and 72 h. The effect of ionizing radiation on the increase of glycolytic metabolism was recently described in cultured tumour cells (Fujibayashi et al., 1997).

A further group is formed with two enzymes that are engaged in the detoxification process. ALDR exhibits a broad substrate specificity for many endogenous and xenobiotic carbonyl compounds. The upregulation of ALDR can reflect the accumulation of both saturated and

Table 1. The list of 28 identified proteins exhibiting significant radiation-induced abundance alterations. Proteins were annotated by MALDI-TOF MS; LC-MS/MS was applied only in the case of PDI, A6 precursor.

Protein name	Accesion No.	Mr[kDa] / pI theoretical	Mr[kDa] / pI measured	Sequence coverage
RHO GDP-dissociation inhibitor 1 [a)]	Q99PT1	23.4/5.1	29.3/4.9	35.3 %
RHO GDP-dissociation inhibitor 2 [a)]	Q61599	22.9/4.9	28.2/4.7	63.0 %
Ras-related protein Rab 1A [a)]	P11476	22.7/5.9	17.0/5.9	23.0 %
14-3-3 protein zeta/delta [a)]	P35215	27.8/4.7	31.9/4.5	17.1 %
Histidine triad nucleotide-binding protein [a)]	P70349	13.9/6.4	18.0/5.8	52.0 %
Transketolase [a)]	P40142	61.1/6.5	60.9/6.8	25.0 %
Pyruvate kinase, M2 isozyme [a)]	P52480	57.8/7.4	54.2/6.7	13.6 %
Aldose reductase [a)]	P45376	36.1/6.7	36.6/6.5	44.0 %
T-complex protein 1, epsilon subunit [a)]	P80316	59.6/5.7	57.0/5.5	33.1 %
Protein disulphide isomerase A6 precursor [a)]	Q63081	47.2/5.0	49.7/4.8	17.2 %
Nucleoside diphosphate kinase B [a)]	Q01768	17.4/7.0	20.8/6.0	27.6 %
Vascular endothelial growth factor D [a)]	P97946	40.9/6.3	38.6/6.0	31.3 %
Inosine-5'-monophosphate dehydrogenase 2 [a)]	P24547	56.2/6.9	53.9/6.1	13.0 %
Translationally controlled tumour protein [a)]	P14701	19.5/4.8	25.4/4.6	30.2 %
40S Ribosomal protein SA [a)]	P14206	32.7/4.7	41.6/4.5	19.0 %
Initiation factor 5A [a)]	P10159	15.6/4.3	20.7/4.8	58.2 %
26S Proteasome regulatory subunit S12 [a)]	P26516	36.5/6.3	39.0/6.0	28.7 %
Metal-response element-binding transcription factor 2 [a)]	Q02395	42.0/8.1	54.3/6.0	15.0 %
Epididymal 17 kDa protein precursor [a)]	AAK58105	20.0/5.7	21.3/5.4	13.0 %
(L04851) putative protein [a)]	AAA40528	26.0/7.9	26.7/6.8	19.0 %
2610008O03RIK protein [a)]	Q9CV92	51.3/8.9	52.4/6.0	38.5 %
2700049I22RIK protein [a)]	Q9CQ99	11.7/4.4	19.2/4.3	69.6 %
2610511I09RIK [a)]	Q9CQ38	47.0/6.9	45.5/6.1	25.9 %
Peroxiredoxin 4 [a)]	O08807	31.3/6.7	30.5/5.7	24.0 %
Protein disulphide isomerase A6 precursor [b)]	Q63081	47.2/5.0	49.3/4.9	24.8 %
Proteasome subunit, alpha type 6 [b)]	Q9QUM9	27.8/6.3	30.8/5.8	21.0 %
T-complex protein 1, beta subunit [b)]	P80314	57.4/5.9	53.2/5.7	51.6 %
26S Proteasome, non-ATPase subunit [b)]	O35593	34.6/6.2	34.6/5.7	24.3 %

a) Proteins whose radiation-induced abundance alterations exhibited either a positive or a negative fold change with magnitude of ≥ 2.

b) Proteins whose radiation-induced abundance alterations exhibited a statistically significant either positive or negative fold change with magnitude of < 2.

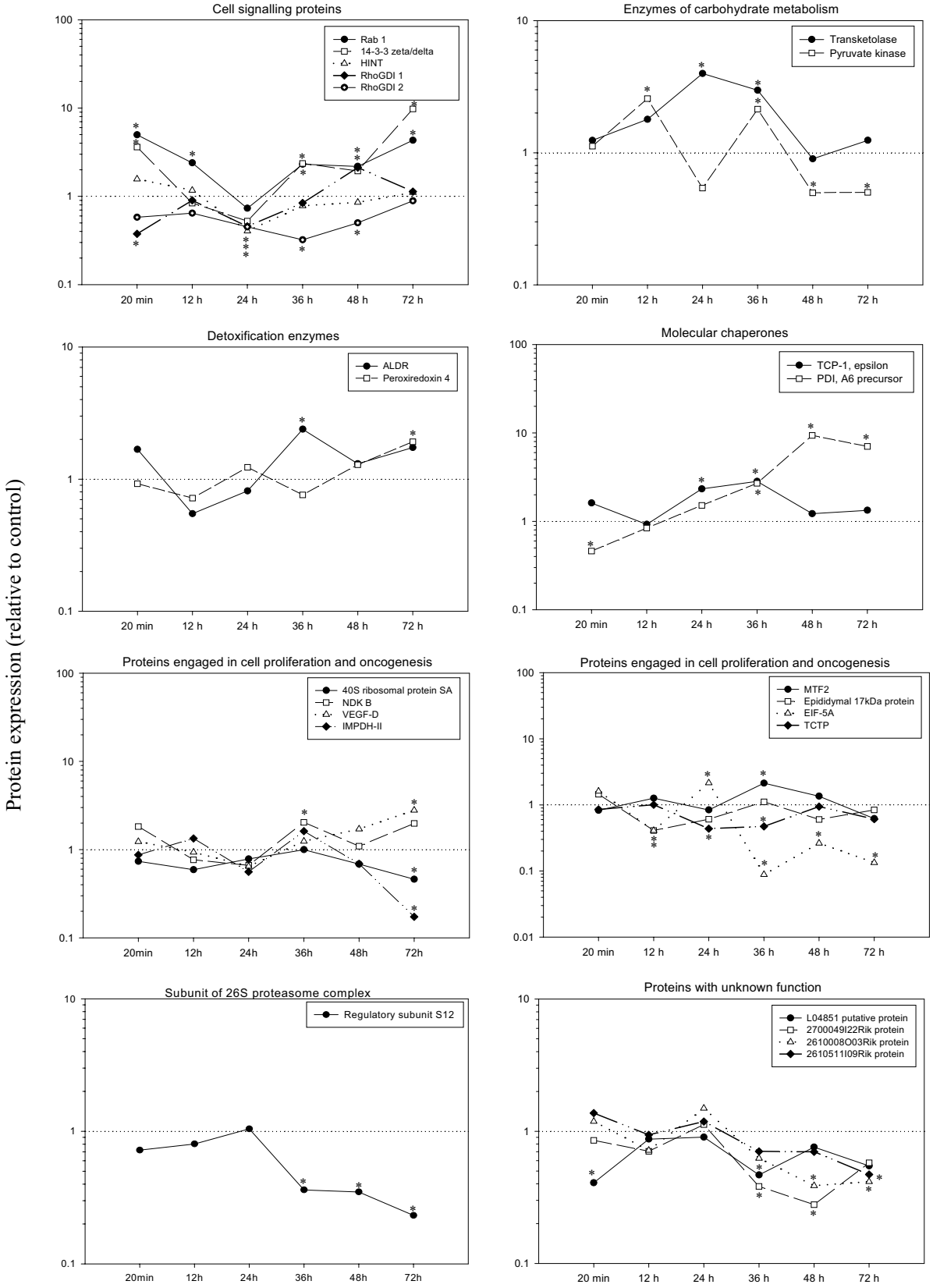

Fig. 2. Radiation-induced abundance alterations of identified 24 proteins in the course of 72 h. Each data point represents the mean values of relative spot intensities from two independent experiments (N = 2). Asterisks indicate the protein levels exhibiting a radiation-induced 2-fold increase or decrease.

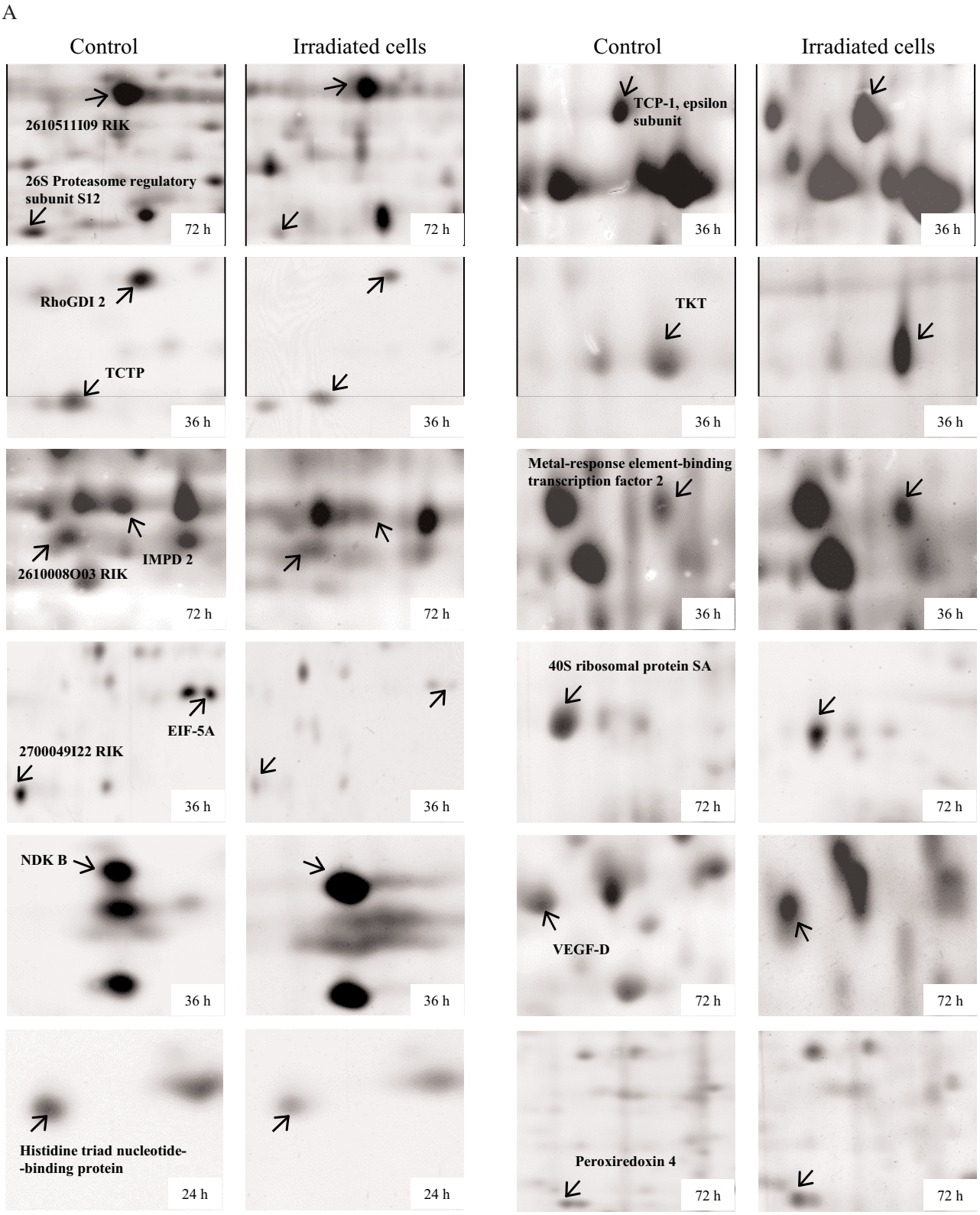

Fig. 3. The sections of two-dimensional images showing differences in protein expressions between control (left panel) and irradiated L929 cells (right panel) in the late (A) or early (B) time intervals. The position of protein spots exhibiting significant 2-fold abundance alterations is denoted with arrows in selected sections.