

## Supplementary data – DIGE Experiment

### **Material and methods -**

#### **2D-DIGE sample preparation and mass spectrometry analysis**

After precipitation and solubilization in lysis buffer, the labeling was carried out by incubation of 400 pmol of fluorophore for 50 µg of protein adjusted to a concentration between 2.5 and 10 µg.L<sup>-1</sup>, on ice and in the dark for 30 minutes. The cyanine NHS ester solutions were reconstituted with 12.5 µl of dimethylformamide (anhydrous DMF) to 5 nmol of fluorophore to obtain a concentration of 400 µmol.mL<sup>-1</sup>. The labeling reaction was then stopped by addition of lysine at 10 mmol.L<sup>-1</sup>. 19.6 µl of each protein sample (concentrated at 2.6 µg.l<sup>-1</sup>) (24 in total) was labeled with 1.2 µl of cyanine fluorophore NHS, either cyanine3-NHS or cyanine-NHS. The proteins constituting the internal standard (196 µL) were labelled by the addition of 12.2 µL of fluorophore cyanine2-NHS. For the constitution of samples groups, a procedure called "dye swap" was used to increase the robustness of 2D-DIGE analysis. After the labeling, samples were assembled. To each solution were added 60 µL of sample dilution buffer (4M Urea, 2M Thiourea, 1% CHAPS, 1% ASB14, 10% glycerol, 1.2% HED, bromophenol blue) previously supplemented with IPG in order to obtain 0.75% of IPG 3-11 ampholytes in the sample. The IEF strips (immobilin dry strip, pH 3-11 NL 24 cm, GE Healthcare) were rehydrated at night using 450µL of rehydration solution (4M Urea, 2M Thiourea, 1% CHAPS, 1% ASB14, 10% glycerol, 1.2% HED, bromophenol blue) containing 0.75% IPG pH 3-11. 120µL of protein samples were deposited on the strip using a cup system and IEF was performed using IPGphor (Ettan IPGphor IEF system, GE Healthcare) during 20h, at 15 °C with a maximum of 50 µA by strip. Strips were then incubated for 15 minutes in the dark in equilibration buffer containing 1% DTT (4M Urea, 2M Thiourea, 1% CHAPS, 1% ASB14, 30% glycerol, 75mM Tris, 2% SDS, bromophenol, pH 8.8). Then, strips were incubated for an additional 15 minutes and in the dark in the equilibration buffer containing 2.5% iodoacetamide. Strips were then deposited on the gel (acrylamide gels 24cm to 12% (Acrylamide-bisacrylamide 37.1: 1)) and sealed with a 0.5% agarose solution. Protein migration took place for 20h at 1.5Watt per electrophoresis tank gel (Ettan6 GE Healthcare) in the dark. The

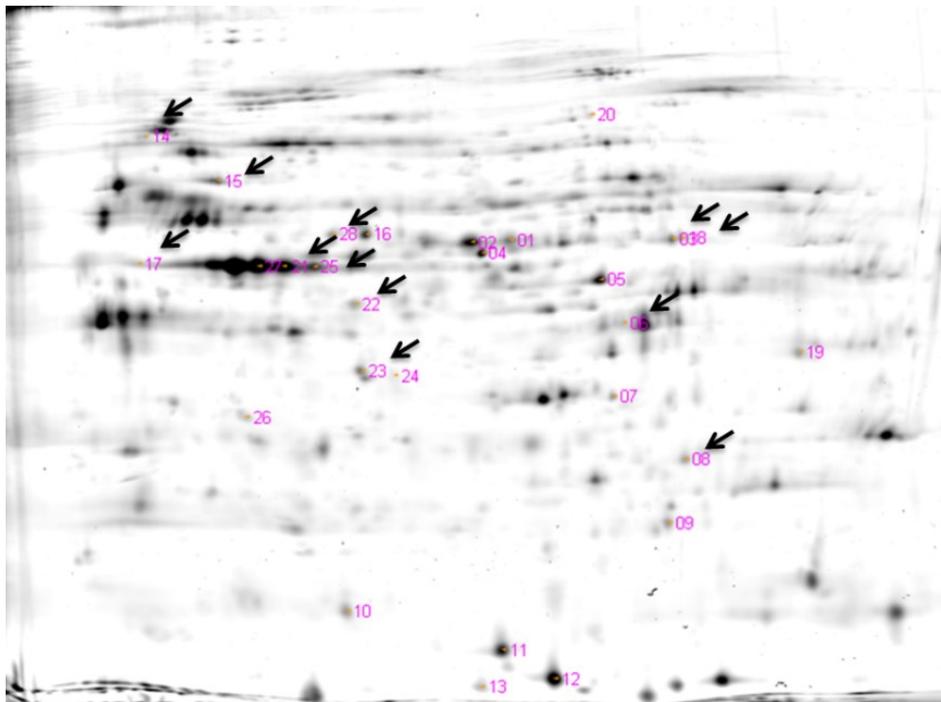
migration took place at 12 ° C thanks to a MultiTemp IV (GE Healthcare) thermostatic circulation system that maintains the migration buffer temperature (TGS). To avoid depletion of electrolytes, 2x buffer is used for the upper chamber. After SDS-PAGE, cyanine dye-labeled protein gels were scanned directly using Typhoon FLA 9500 (GE Healthcare) at a resolution of 50 µm. After fluorescence imaging, gels were washed for 10 min in ultrapure water and post-stained with the Imperial™ Protein Stain (Thermo Scientific, Rockford, USA). The Samespot software package (version 4.0.3; nonlinear dynamics) was used to determine protein abundance and statistics based on 2D-DiGE. Based on Samespot analysis, spots of interest were excised using a Shimadzu Biotech Xcise System (Champs sur Marne, France) and submitted to trypsin digestion after reduction and alkylation. The different peptides were then characterized by mass spectrometry (MALDI-Tof MS / MS UltraFlexIII Bruker Daltonics, and Orbitrap LTQ ThermoFischer Scientific velos) and proteins were identified by interrogation of the NCBI nr / C.elegans databases and Uniprot / UPP\_C.elegans. The MALDI TOF / TOF was used for a first identification, refined by the use of Orbitrap.

### **Enrichment analysis**

Following the differential identification of proteins expressed after irradiation in *C. elegans*, an analysis of biological functions was performed using the DAVID gene interaction tool [23]. Indeed, the proteins have been grouped according to the biological processes that they govern (GOTERM) for a simplified analysis. After selection of the model organism (*C. elegans*), the UniProt accession number of the differently expressed major proteins were implemented in the David gene tool, as well as the background noise (all the majority proteins identified in our study). Then, the biological processes, in which the variant proteins were involved between the different irradiation conditions, were searched and classified according to their pvalue (ease value = 1, because of the number of modulated proteins)

## Results -

The analysis of the differential protein expression by the 2D-DIGE methodology after chronic irradiation of *C. elegans* at 0.5, 1 and 3.3 Gy showed that among the 937 spots detected, only one was considered differently expressed compared to control conditions (**Figure A**). Mass spectrometry analysis revealed that this spot corresponds to actin-1 and actin-4 (accession numbers P0DM41 and P10986 respectively); 2 cytoskeleton proteins which are also expressed in the *C.elegans* reproductive system, and especially in the germcell precursors (Z2 and Z3). These proteins are significantly repressed at 1 Gy (ratio of 0.67,  $p$ . value =0.032) compared to control condition, showing that mis-regulation of protein expression occurs from 1 Gy of chronic exposure to gamma rays.



**Figure A:** 2D-DIGE electrophoresis gel. 12 spots of interest after acute and chronic exposure to gamma rays (annotated in pink with arrow), and selected for mass spectrometry analysis. Only the spot n°17 is considered as variant after chronic exposure compared to control conditions.

The analysis of the differential protein expression by the 2D-DIGE methodology after acute irradiation of *C. elegans* at 0.5, 1 and 3.3 Gy has shown that among the 937 detected spots, 12 were significantly observed as variants compared to control conditions (**Figure A**). Thus, among the 12 differential protein spots, 15 proteins have been identified. In contrast, 2 spots (n°8 and n°23) could not be identified by mass spectrometry. Results of the 2D-DIGE analysis identification after acute

irradiation are given in table A, and show that protein mis-regulation occurs in acute exposure from 0.5 Gy of gamma rays.

Enrichment analysis after the 2D-DIGE methodology was difficult to perform because of the low number of identified proteins, but this analysis showed that mis-regulated proteins after acute exposure, i.e. RACK-1, ACT-1, ACT-5, HSP-1 and RPS 0, are involved in biological pathways such as “larval development” (100% proteins, p. value = 0.13); “gonad development” (67% proteins, p. value = 0.18); “reproductive process” (78% proteins, p. value = 0.25); “embryo development ending in birth or egg hatching” (100% proteins, p. value = 0.38) or “embryo development “ (100% proteins, p. value = 0.38) (**Figure B**). Excepted RACK-1 which is required during the cytokinesis phase and during germline cell membrane organization in *C. elegans* [27], these proteins are not specific to the reproduction.

It is important to note that proteins analyzed as mis-regulated after 2D-DIGE analysis and label free LC-MS/MS methodology are different because of i) exclusion of some proteins from the 2D gel (hydrophobic proteins for example or proteins which have molecular weight <5kDa and> 150Kda, or low abundance proteins) and ii) the difference of quantitative proteomic processing between the two methodologies. Indeed, quantitative method was much more stringent for the label free LC-MS/MS method (fDR 1% using 250 permutations; proteins represented at minimum 3 replicates of 6 of a same condition), and this could explain why protein analyzed as mis-regulated by the 2D-DIGE methodology were not found significantly mis-regulated by the label free LC-MS/MS method.

Finally, results of the 2D-DIGE are consistent with the label free based LC-MS/MS approach as biological processes such as “embryo development ending in birth or egg hatching” and “embryo development” were also found to be mis-regulated after acute exposure, attesting of the reprotoxic nature of gamma rays [4], [17]. Moreover, biological pathways such as “larval development” and “post-embryonic development” are also common between the 2D-DIGE method and the label free

method. This comparison between the two methodologies proves that regulation processes are similar and finally attest of the robustness of our results.

Table A: List of mis-regulated proteins after acute irradiation on *C. elegans* from 0.5 to 3.3 Gy compared to control conditions and identified by mass spectrometry (Maldi-TOF/TOF and Orbitrap). Protein expression modulation compared to control condition is given for each of the identified protein. A ratio inferior to 0.7 means that the protein spot is repressed compared to control conditions, conversely, a ratio superior à 1.5 means that the protein spot is overexpressed compared to control conditions (\*P<0.05; \*\*P<0.01; \*\*\*P<0.001, Student test – DECODON DELTA 2D Software).

Modulation						N° spot	Protein ID	ID Gene	Uniprot accession number	Score	Sequence coverage (%)	Molecular weight (Da)
0.5 Gy Vs. control		1 Gy Vs. Control		3.3 Gy Vs. control								
Fluorescent Intensity ratio (volume)	<i>p.value</i>	Fluorescent Intensity ratio (volume)	<i>p.value</i>	Fluorescent Intensity ratio (volume)	<i>p.value</i>							
0.72	0.003**	0.59	0.012*			3	Probable fumarate hydratase, mitochondrial	fum-1	O17214	1789	49	53784
0.72	0,003**	0.59	0.012*			3	Probable citrate synthase, mitochondrial	cts-1	P34575	964	33	51793
				2.22	0.047*	6	Guanine nucleotide-binding protein subunit beta-2-like 1	rack-1	Q21215	841	40	36150
		2.50	0.003**			14	Heat shock 70 kDa protein A	hsp-1	P09446	1662	46	69965
0.70	0.046*			0.66	0.026*	15	Chaperonin homolog Hsp-60, mitochondrial	hsp-60	P50140	2585	45	60235
2.08	0.006**	2.16	0.035*			17	Actin-1	act-1	P0DM41	1176	40	42111
2.08	0.006**	2.16	0.035*			17	Actin-4	act-4	P10986	99	47	42093
0.66	0.001**					18	Probable fumarate hydratase, mitochondrial	fum-1	O17214	384	22	53784
				1.56	0.001**	21	Actin-1	act-1	P0DM41	5934	52	42111
				1.56	0.001**	21	Actin-5	act-5	O45815	3239	58	42131
		1.55	0.049*			22	40S ribosomal protein SA	rps-0	P46769	871	55	30798
		1.55	0.049*			22	Elongation factor 2	eef-2	P26691	538	16	95477
				1.66	0.001**	25	Actin-1	act-1	P0DM41	1121	52	42111
				1.66	0.001**	25	Actin-5	act-5	O45815	1068	58	42131
0.71	0.009**			0.72	0.015*	28	Enolase	enol-1	Q27527	396	28	46759

