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Intracellular Signal Transduction in Mouse Oocytes and Irradiated Early Embryos

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Abstract. *In order to determine the effect of X-irradiation on intracellular signal transduction in mouse oocytes and embryos, JNK, ERK and p38 kinase activities were measured by the state of phosphorylation of their respective substrates (c-Jun, Elk-1 and ATF-2, respectively) in two mouse strains differing in radiation sensitivity, namely C57BL and BALB/c. In a first step, control oocytes and embryos were compared for their respective kinase activities at various stages of oocyte maturation (germinal vesicle and metaphases of 1st and 2nd meiosis stages) and early embryonic development (1-, 2-, 4-, 8- and 16-cell, morula and blastula stages). Levels of p38, ERK or JNK kinase activities were shown to vary with the stage of oocyte maturation and embryo development. In a second step, 1- and 2-cell embryos were X-irradiated with 2.5 Gy during the S-phase of the 1st or the 2nd cell-cycle, respectively. There were no significant differences in p38, ERK and JNK kinase activities between control and irradiated embryos, whatever the stage or mouse strain was considered. In conclusion, p38, ERK and JNK kinase activities were shown to vary during oocyte maturation and early embryonic development. Apparently, X-irradiation did not affect these kinase activities at the 1- and 2-cell stages in either mouse strains regardless of their difference in radiation sensitivity.*

The mechanisms of signal transduction in oocytes and in early embryos are not fully understood. In mammalian germ cells, gene transcription ceases with oocyte maturation and is switched on again during the second cell-cycle after fertilization (or later, depending on the species). Therefore, signal transduction analysis in the oocyte and in the early embryo of the mouse represents a unique opportunity to study signal transduction in mammalian cells with or without an actively transcribing genome, respectively. Since preimplantation development is remarkably similar among all mammalian species studied so far, it may be assumed that the molecular regulation of cell-cycle control is similar in human and mouse preimplantation embryos and that the conclusions obtained from studies on mouse embryos may be of interest for human embryology.

Our laboratories, as well as others, have shown that BALB/c mice are more susceptible to ionising radiation than C57BL mice, in particular with regard to cell-cycle arrest of one-cell embryos and radiation-induced mammary cancer (1-3). One-cell embryos of certain mouse strains are particularly sensitive to radiation-induced G2-arrest (1). The sensitivity of the embryo to this effect is determined by the maternal genotype and, as we have previously shown, the BALB/c strain is more radiation sensitive than the C57BL strain. Interestingly, the outstanding sensitivity of the BALB/c embryo to radiation-induced G2-arrest seems to be limited to the one-cell stage, since irradiation at later stages is much less effective.

Forrer *et al.* (4) have established a rapid, non-radioactive, colorimetric ELISA assay for enzyme activity measurements of Jun-N-terminal (JNK), p38 and ERK kinase activities (4) (for principles see Figure 1). The assay takes advantage of commercially available polyclonal antibodies recognising phosphorylated epitopes of the kinase substrates c-Jun (Ser73), ATF-2 (Thr 71) and Elk1. The kinase substrates are genetically engineered fusion proteins with glutathione-S-transferase (GST) or 6xHis-tagged-phage lambda protein D.

In this study, kinase activities were detected in crude extracts from 40 pooled oocytes or embryos. We measured the activities, with and without 2.5 Gy X-irradiation, of the kinases p38, ERK and JNK in oocytes,

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Key Words: Oocyte, embryo, mouse, signal transduction, irradiation, Elk-1, c-Jun, ATF-2.

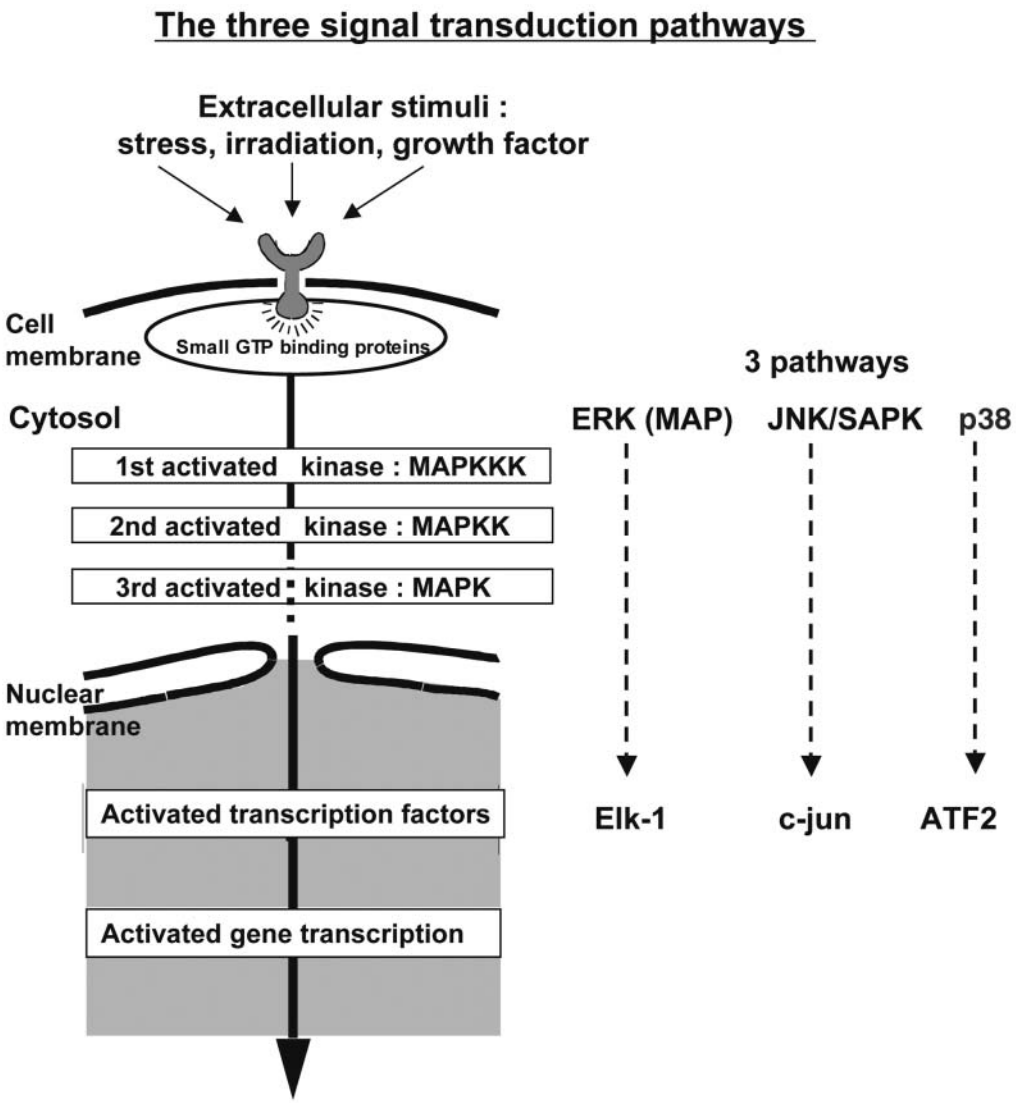

Figure 1. *Schematic representation of the signal transduction pathways of interest in this paper: ERK, JNK and p38 kinases phosphorylate Elk-1, c-Jun and ATF-2, respectively.*

as well as in embryos from fertilization to the blastula stage and compared the corresponding values in two mouse strains (BALB/c and C57BL) with markedly different radiation sensitivities.

Materials and Methods

Oocyte and embryo collection. Female BALB/c and C57BL mice aged 10-12 weeks were purchased from B&K (Hull, United Kingdom). The mean weight of the females at the time of the experiment was around 30 g. Between 30 and 40 animals per X-irradiation condition and per mouse strain were used for these experiments. In order to collect cells at the germinal vesicle (GV) stage and at the metaphase of first meiosis (MI), oocytes were released from large antral follicles of BALB/c and C57BL females. Immature (GV) oocytes were isolated and frozen immediately in liquid nitrogen to prevent spontaneous germinal vesicle breakdown. Some GV oocytes were cultivated at 37˚C in an atmosphere of 5% CO_2 in air and in Yamada's medium (5) containing 0.01 mM EDTA and 250 ng/ml of colchicine (Gibco BRL, Merelbeke, Belgium) in order to block them in metaphase of the 1st meiosis (MI). Oocytes that reached the MI stage were then frozen in liquid nitrogen.

To obtain oocytes in metaphase of the second meiosis (MII oocytes), BALB/c and C57BL females were induced to superovulate by intraperitoneal injection of 5 IU pregnant mare serum (PMS, Folligon®, Intervet, Beaucouse, France) followed 45-48 h later by 5 IU human chorionic gonadotrophin (hCG, Pregnyl®, Organon, Brussels, Belgium) (6, 7). Superovulation occurs at 12±3 h after hCG injection. Ovulated (MII) oocytes in meiosis II were collected by rupture of the ampulla tubae at 15 h post-hCG and by harvesting in Yamada's medium (5) containing 0.1% hyaluronidase (Sigma, Bornem, Belgium) to disperse follicular cells.

To obtain embryos, females were individually caged with a male of the same strain from 15 to 17 h after hCG injection. They were examined immediately thereafter for the presence of a vaginal plug. Fertilization of the positive females was considered to have occurred at the middle of this short mating period. Embryos were flushed out from the ampulla tubae. Only those which had extruded the second polar body and formed two pronuclei were used for the experiments. They were frozen directly (for 1-cell embryo at the pronuclear stage), or cultivated at 37˚C in an atmosphere of 5% CO_2 in air in Yamada's medium supplemented with EDTA and colchicine (for the 1-cell stage embryo in mitosis) or were allowed to develop up to the second mitosis (2-cell embryo) or even further (4-, 8-, 16- cell; morula or blastula).

X-irradiation at the 1- or 2-cell stage of the embryonic development was performed *in vivo* by placing the pregnant females in a Plexiglas box and by whole-body irradiation (2.5 Gy) with a Pantak HF420 RX machine (Branford, CT, USA) operating at 250 kV, 15 mA, 1 mm Cu filtration and a dose rate of 0.375 Gy/min.

Measurement of p38, ERK and JNK kinase activity. In order to evaluate p38, ERK and JNK kinase activity, the measurement of the phosphorylation of their respective substrates (ATF-2, ELK-1 and c-jun) was performed on pooled samples of 40 oocytes or embryos according to the methodology developed by Forrer *et al.* (4). Embryos were X-irradiated during the S-phase of the first cell cycle or the interphase of the second cell cycle, respectively.

Statistical analysis. Results are expressed as the standard error of the mean (SEM). Student's *t*-test was used for statistical evaluation of the results. Differences were considered to be significant (as shown by *) or highly significant (as shown by **) if the *p* value was less than 0.05 or 0.001, respectively.

Results

Signal transduction pathways in control cells. Because of the lack of information available on the signal transduction pathways in 1 and 2-cell mouse embryos, the first experiments were devoted to the comparison between different stages of maturation of oocytes and early stages of embryonic development. For all experiments, two mouse strains (BALB/c and C57BL) with different radiosensitivities were compared. Figure 2 shows p38, ERK and JNK kinase activities during oocyte maturation and early embryonic development.

The level of p38 kinase activity was clearly higher during meiotic (MI and MII) and mitotic divisions than during interphase (GV oocytes, 1-cell embryos at the pronuclear stage or 2-cell embryos at the interphase stage). It was also low at the 4-, 8-, 16- and 32-cell stage but increased with the size of the embryo (4-cell embryo <8-cell embryo <16-cell embryo <32-cell embryo). When comparing the same stage of development between the BALB/c and C57BL mouse strains, no significant differences could be observed except for oocytes in metaphase of 1st meiosis that gave a higher p38 activity in the C57BL strain (Tables I and II).

The ERK kinase activity increased drastically during metaphases of the two meioses (MI and MII oocytes) and decreased to low values during all stages of early embryonic development (Figure 2). Only MI oocytes appeared to display a minor strain-to-strain difference (C57BL>BALB/c).

In contrast to p38 and ERK activities, the level of JNK activity was highest in GV oocytes and decreased continuously during maturation (MI and MII). Low values of activity were found in embryos; small but increasing values were recorded from the morula stage onwards (Figure 2, Table I). During all stages, no significant strain-to-strain differences were observed for JNK activity (Table II).

Signal transduction in X-irradiated cells. 1- and 2-cell embryos were X-irradiated with 2.5 Gy during the S-phase of the first cell-cycle or the interphase of the second cell-cycle, respectively. Embryos were collected during "normal" (for those escaping the block) or "delayed" mitosis as well as during G2-arrest.

As illustrated in Figure 3 and Table I, there were no significant differences in p38, ERK and JNK kinase activities between control and irradiated embryos, whatever the stage considered in both mouse strains. Furthermore, we did not find any significant differences between the two mouse strains tested (Table II).

Discussion

The analysis of signal transduction in mouse oocytes and embryos provides the special opportunity to study irradiation effects in a system with or without active transcription of the genome. Oocytes stop transcription and will only resume it after fertilization, in the late second cell-cycle. Therefore, there is a period of about 48 h in which no transcription takes place. To record signal transduction during this time, we determined the effects of irradiation on the kinase activities of p38, JNK and ERK in mouse oocytes and early embryos.

MAP kinases, including ERK, are known to be activated during meiotic maturation in Xenopus, starfish and mouse oocytes and have an essential role in both transitions from G2- to the M-phase of meiosis and metaphase arrest of mature oocytes. However, as reported by Takenaka and colleagues (8), little is known about the role of MAP/ERK in the M-phase of somatic cells of the early embryo. p38 kinase but not MAP/ERK or JNK is activated in somatic cells blocked in M-phase by nocodazole (8). This observation is in line with the high levels of p38 kinase activity observed in our embryos during mitosis, as well as with the very low levels of ERK and JNK activities at all stages (mitosis or interphase).

According to Villareal and colleagues, p38 kinase activity could have an important function in early development (9). Indeed, ATF-2 RNA and protein levels

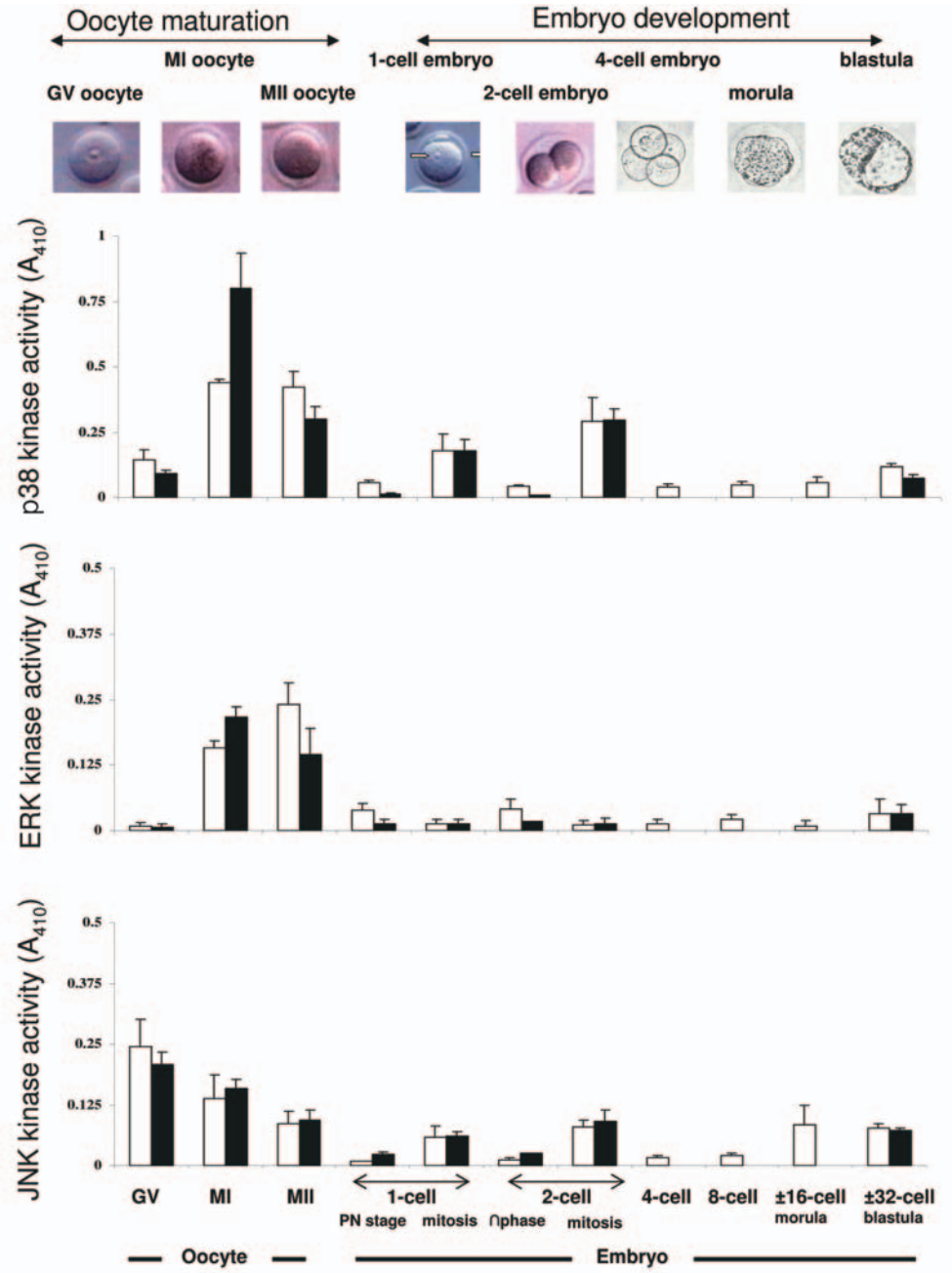

Figure 2. *p38, ERK and JNK kinase activities during oocyte maturation and early embryonic development. White and black columns represent the mean values from 3 separate experiments (± standard error of the mean) in the BALB/c and C57BL strains, respectively, and as described in Materials and Methods (GV oocytes: oocytes arrested in late prophase of the first meiotic division; MI: oocytes in metaphase of the first meiotic division; MII: ovulated oocytes in metaphase of the second meiotic division; ∩ phase: interphase).*

were very low in Xenopus oocytes, but rose dramatically during blastulation. We observed only a small increase of p38 kinase activity in embryos from the 4-cell stage to the blastocyst stage.

Our results did not reveal any response of p38 kinase, ERK or JNK activities to 2.5 Gy X-irradiation of 1- or 2-cell embryos. Analyses of the kinase activities were essentially performed around 12 h after irradiation, during the period of cell-cycle arrest. Since the activation of the kinases can be rapid and transient, it will be important to test earlier time points. Such experiments demanding even more extensive studies with animals are in progress.

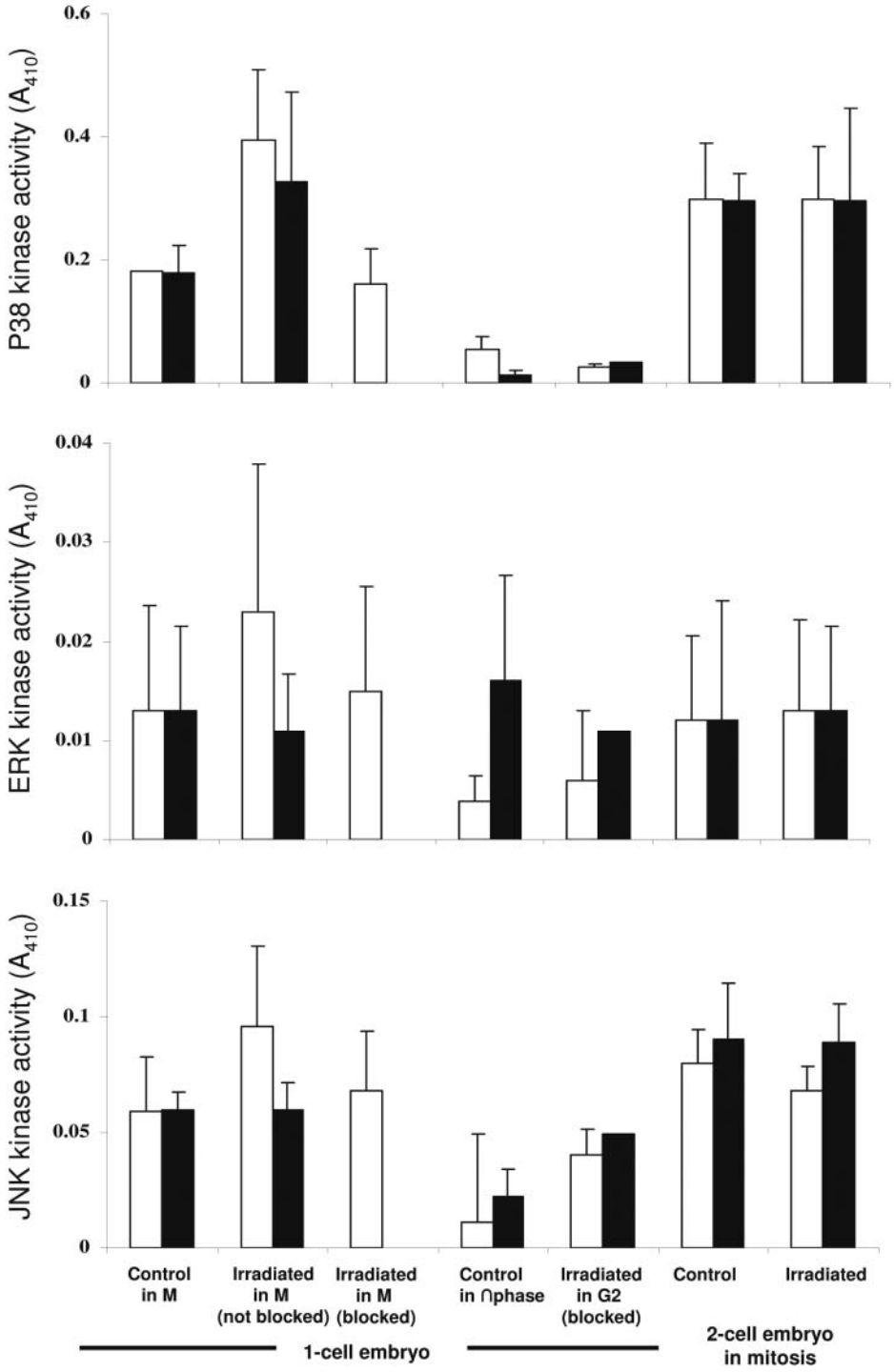

Figure 3. *p38, ERK and JNK kinase activities in control and X-irradiated embryos at the 1- or 2-cell stage. White and black columns represent the mean values from 3 separate experiments ± SEM in the BALB/c and C57BL strains, respectively, and as described in Materials and Methods.*

p38, ERK and JNK kinase activities were shown to vary during oocyte maturation and early embryonic development. Apparently, X-irradiation did not affect these kinase activities at the 1- and 2-cell stages in either mouse strain regardless of their difference in radiation sensitivity.

Table I. *Statistical comparison of p38, ERK and JNK kinase activities between various stages of development in control and irradiated conditions of BALB/c and C57BL mouse strains (*p<0.05; **p<0.001; NS, not significant; vs., versus; ctrl, control; irr, irradiated).*

	BALB/c			C57BL		
	p38	ERK	JNK	p38	ERK	JNK
Control conditions (Figure 2)						
Germinal vesicle stage *vs.* metaphase I oocytes	**	**	NS	*	**	NS
Germinal vesicle stage *vs.* metaphase II oocytes	*	*	*	*	*	*
Metaphase I *vs.* metaphase II oocytes	NS	*	NS	*	NS	*
Metaphase II oocytes *vs.* 1-cell embryo at pronuclear stage	*	*	NS	*	*	*
Metaphase II oocytes *vs.* 1-cell embryo in mitosis	*	*	NS	NS	*	NS
1-cell embryo interphase *vs.* 1-cell embryo in mitosis	NS	NS	NS	*	NS	*
1-cell embryo in mitosis *vs.* 2-cell embryo in mitosis	NS	NS	NS	NS	NS	NS
1-cell embryo in mitosis *vs.* 2-cell embryo in interphase	*	NS	NS			
1-cell embryo in interphase *vs.* 2-cell embryo in interphase	NS	NS	NS			
2-cell embryo in mitosis *vs.* 4-cell embryo	*	NS	*			
4-cell embryo in mitosis *vs.* 8-cell embryo	NS	NS	NS			
8-cell embryo in mitosis *vs.* 16-cell embryo	NS	NS	NS			
16-cell embryo in mitosis *vs.* 32-cell embryo	NS	NS	NS			
Irradiated conditions (Figure 3)						
ctrl *vs.* irr (not blocked) 1-cell embryo in mitosis	NS	NS	NS	NS	NS	NS
ctrl *vs.* irr (+ blocked) 1-cell embryo in mitosis	NS	NS	NS			
ctrl *vs.* irr 1-cell embryo in interphase	NS	NS	NS			
ctrl *vs.* irr 2-cell embryos in mitosis	NS	NS	NS	NS	NS	NS

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Table II. *Statistical comparison of p38, ERK and JNK kinase activities between BALB/c and C57BL mouse strains at the different stages of oocyte maturation and early embryonic development (*p<0.05; **p<0.001; NS, not significant; irr, irradiated).*

	BALB/c *versus* C57BL		
	p38	ERK	JNK
Oocytes at germinal vesicle stage	NS	NS	NS
Oocytes in metaphase I	*	*	NS
Oocytes in metaphase II	NS	NS	NS
1-cell embryo in mitosis	NS	NS	NS
1-cell embryo in interphase	NS	NS	NS
2-cell embryo in mitosis	NS	NS	NS
32-cell embryo	NS	NS	NS
irr 1-cell embryo in mitosis (not blocked)	NS	NS	NS
irr 1-cell embryo in interphase (blocked)	NS	NS	NS
irr 2-cell embryo in mitosis	NS	NS	NS

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