

Cross-linking of Qa-2 protein, the *Ped* gene product, increases the cleavage rate of C57BL/6 preimplantation mouse embryos

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The Qa-2 protein, a glycosylphosphatidylinositol (GPI)-linked major histocompatibility complex (MHC) Class Ib molecule found on the surface of mouse T-cells and preimplantation embryos, is the product of the preimplantation embryo development (*Ped*) gene. The *Ped* gene regulates the rate of early embryonic development and subsequent embryo survival. T-cells treated with anti-Qa-2 monoclonal antibody (mAb) and cross-linked with a secondary antibody, in the presence of a co-stimulatory signal, undergo increased proliferation. The purpose of this study was to determine whether cross-linking of Qa-2 similarly affects preimplantation embryos. We cross-linked Qa-2 protein on the surface of C57BL/6 2-cell and 8-cell embryos, in the presence of 4/5-phorbol-12-myristate-13-acetate (PMA), and assessed the percentage of embryos reaching the blastocyst stage, the percentage hatching from the zona pellucida, [³H-thymidine] incorporation into DNA, and the total number of cells per embryo as measures of embryonic cleavage rate. Both 2-cell and 8-cell embryos increased their cleavage rates 48 h after cross-linking of Qa-2, compared with control embryos ($P < 0.05$). Our results indicate that a Qa-2 protein cross-linking mechanism may be one way by which this protein regulates the rate of preimplantation mouse embryo development.

Key words: MHC/*Ped* gene/preimplantation embryo development

Introduction

The major histocompatibility complex (MHC) is a cluster of genes found in all mammalian species. Many of these genes encode proteins that have a defined role in regulating the immune response. For instance, Class Ia MHC molecules, encoded by the K and D regions of the mouse MHC, are polymorphic, ubiquitously expressed transmembrane glycoproteins that present endogenously derived peptides to CD8⁺ T-cells. The majority of mouse Class I MHC molecules, however, are the Class Ib proteins, encoded by the Q and TL regions of the H-2 complex. Although structurally similar to Class Ia molecules, Class Ib proteins are less polymorphic and more restricted in their tissue expression (reviewed in Stroynowski, 1990; Stevens and Flaherty, 1996; Stroynowski and Tabaczewski, 1996). The functions of most Class Ib molecules are unknown.

Qa-2 protein, a Class Ib molecule, is encoded by four similar tandem genes, *Q6*, *Q7*, *Q8*, and *Q9*, in the Q region of the H-2 complex (Cai *et al.*, 1996). The *Q6* and *Q8* genes are very similar and are referred to as the *Q6/Q8* gene pair, while the *Q7* and *Q9* genes are referred to as the *Q7/Q9* gene pair. The Qa-2 protein encoded by the *Q7/Q9* gene pair is linked to the cell surface by a glycosylphosphatidylinositol (GPI) anchor and, therefore, is restricted to the outer leaflet of the plasma membrane. Like several other GPI-linked molecules, Qa-2 protein is believed to be involved in cell signalling because monoclonal antibodies (mAb) to Qa-2, when cross-linked by a secondary antibody in the presence of a

co-stimulatory molecule, cause T-cell activation (Hahn and Soloski, 1989; Robinson *et al.*, 1989; Cook *et al.*, 1992; Hahn *et al.*, 1992; Horejsi *et al.*, 1999). The mechanism of this activation pathway is not yet known, although mAb binding to Qa-2 results in rapid tyrosine phosphorylation of specific substrates and an influx of intracellular calcium (Robinson and Hederer, 1994). Cell proliferation then can be induced by a co-stimulatory signal such as that provided by 4/5-phorbol-12-myristate-13-acetate (PMA) (reviewed in Robinson, 1997). Importantly, the GPI-linkage is required for Qa-2-mediated signalling (Robinson *et al.*, 1989).

Qa-2 protein also is involved in regulating early mouse embryonic development. Qa-2 is expressed on the surface of mouse preimplantation embryos beginning at the 2-cell stage (Warner *et al.*, 1987; McElhinny and Warner, 1997) and continuing through the blastocyst stage where it is expressed on both the inner cell mass and the trophectoderm (McElhinny *et al.*, 1998). Qa-2 protein is the product of the *Ped* gene (Xu *et al.*, 1994; Wu *et al.*, 1999). The *Ped* gene has two functional alleles: the dominant *Ped fast* allele is the presence of the Qa-2 protein on the embryonic cell surface while the recessive *Ped slow* allele is the absence of Qa-2 protein (Warner *et al.*, 1987). *Ped fast* preimplantation embryos develop at a significantly faster rate, both *in vivo* and *in vitro*, compared with *Ped slow* embryos (Warner *et al.*, 1987; Brownell and Warner, 1988). *Ped fast* mouse strains also exhibit an overall survival advantage during gestation (Warner *et al.*, 1993; Exley and Warner, 1999), and have larger litter sizes and heavier body weights at birth and weaning (Warner *et al.*, 1991). The

Ped gene phenotype, fast and slow preimplantation development, exists in humans, but the human homologue of the mouse *Ped* gene has not yet been identified (Cao *et al.*, 1999).

Although Qa-2 protein is encoded by four similar genes, *Q6*, *Q7*, *Q8*, and *Q9*, only two, *Q7* and *Q9* (one nucleotide difference), are expressed in preimplantation mouse embryos. *Ped fast* mouse embryos express the *Q7* gene, the *Q9* gene, or both, depending on the strain (Wu *et al.*, 1998). *Ped slow* embryos have a deletion of both the *Q7* and *Q9* genes. Embryonic Qa-2 is GPI-linked to the cell surface of mouse embryos (Tian *et al.*, 1992). Removal of the Qa-2 protein by phosphatidylinositol phospholipase C (PI-PLC), an enzyme that cleaves GPI-anchors, changes the phenotype of *Ped fast* embryos to *Ped slow* (Tian *et al.*, 1992). Additionally, antisense oligonucleotides to mRNA transcripts from the *Q7* and *Q9* genes decrease the cleavage rate of *Ped fast* embryos (Xu *et al.*, 1993). Conversely, microinjection of either the *Q7* or the *Q9* gene into *Ped slow* zygotes converts the embryos to the *Ped fast* phenotype (Xu *et al.*, 1994, Wu *et al.*, 1999). The mode of action of how Qa-2 functions in development is unknown.

The purpose of this study was to investigate the mechanism of action of Qa-2 protein in regulating the rate of preimplantation embryonic development. We tested the hypothesis that incubation with anti-Qa-2 mAb, followed by cross-linking with a second antibody, in the presence of PMA, would increase the rate of cleavage of the embryos.

Materials and methods

Mice

The H-2^b, *Ped fast* C57BL/6 mouse strain (Qa-2+) was obtained from the Jackson Laboratory, Bar Harbor, ME, USA, and bred in our laboratory. As a negative control, we used B6.K1 mice (*Ped slow*; Qa-2-), obtained from Dr Lorraine Flaherty, Wadsworth Center, Albany, NY, USA, and bred in our laboratory. Mice were housed in a day-night controlled room (lights on 04:00–18:00 h) with food and water *ad libitum*. All experiments followed the NIH guidelines for the care and use of laboratory animals.

Antibodies

Monoclonal antibodies (mAb) were partially purified from ascites fluid by a protein A column (Pierce, Rockford, IL, USA), lyophilized, and frozen at -20°C. Antibodies were titered by FACScan analysis on C57BL/6 T-cell enriched splenocytes before being used for T-cell cross-linking studies. Antibodies used were 1-12-1, a mAb against Qa-2 (Sharrow *et al.*, 1989), 27-11-13, a mAb against the MHC Class Ia H-2D^b protein (Ozato and Sachs, 1981), and TIB109, a control mAb against sheep red blood cells (SRBC) (American Type Culture Collection, Rockville, MD, USA).

T-cell enrichment

C57BL/6 mice were killed by cervical dislocation and the spleens were excised and rubbed over a sterile wire mesh. The tissue fragments were rinsed with 2 ml of Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco BRL, Grand Island, NY, USA) containing 10% heat-inactivated fetal calf serum (FCS; Hyclone, Logan, UT, USA) and 1% antibiotics/antimycotics solution (Gibco BRL) into a 15 ml conical tube. Pieces of connective tissue and other debris were allowed to settle to the bottom of the tube and then the cell suspension

(~35% T-cells) was pipetted onto a pre-equilibrated nylon wool column consisting of 0.6 g of nylon wool loosely packed into a 10 ml syringe attached to a 3-way stopcock (Becton-Dickinson; Rutherford, NJ, USA). The column was incubated for 1 h in a 37°C, 5% CO₂ incubator. The T-cell enriched population (>99% T-cells) was eluted from the column by washing with 20 ml of RPMI 1640. This cell population was >95% viable as determined by Trypan Blue exclusion, and was adjusted to a final concentration of 5×10⁶ cells/ml.

T-cell activation

100 µl of the T-cell enriched population were added to wells of a 96-well plate (5×10⁵ cells/well). Then anti-Qa-2 mAb (1-12-1) or control mAb (27-11-13) was added at various dilutions and the plate was incubated for 30 min at room temperature to allow for antibody binding. Second antibody (50 µg/ml of rabbit anti-mouse IgG; ICN, Costa Mesa, CA, USA) and PMA (5 ng/ml; Sigma Chemical Co, St Louis, MO, USA) in RPMI 1640, or RPMI 1640 only for control wells, were added to designated wells. The plates were incubated for 48 h at 37°C, 5% CO₂ and then the cells were harvested, as described below.

T-cell harvesting

The plate was removed from the incubator 6 h before harvesting, (42 h after cross-linking), and 1 µCi of [³H-TdR] (specific activity = 20 Ci/mmol; NEN-Dupont, Wilmington, DE, USA) in RPMI 1640 was added to each well. The plate was incubated for an additional 6 h. The cells were harvested onto filtermats (Skattron, Lier, Norway) using a Titertek cell harvester connected to a carboy of distilled water. The filters were dried in a vacuum oven at 65°C for 30 min and counted in a liquid scintillation counter. Background values, the amount of [³H-TdR] incorporated by unstimulated T-cells, were subtracted from the activated T-cell experimental values.

Embryo collection

Ovulation was stimulated in 8–12-week old female mice with 5 IU of equine chorionic gonadotrophin (eCG; Sigma) i.p. at the 11th hour of the light cycle. After 48 h, 10 IU of human chorionic gonadotrophin (HCG; Sigma) was injected i.p. and each female was mated with one male of the same strain. On the following morning, the female mice were checked for the presence of a vaginal plug to determine successful mating.

From those mice with plugs, 2-cell embryos were collected 42 h after HCG injection, while 8-cell embryos were collected 66 h after HCG injection. Mice were killed by cervical dislocation and the oviducts were excised and transferred to a glass depression slide (watchglass) containing 0.5 ml of Whitten–Biggers culture medium (WB) under gas. Using a 1 ml syringe with a fine needle (30 g) dulled with a file, embryos were flushed out of the oviducts with WB medium. Morphologically normal embryos were transferred to a clean depression slide containing 0.5 mg/ml, or otherwise noted concentrations, of monoclonal antibodies 1-12-1, 27-11-13, or TIB109 in WB, in a total volume of 200 µl. Embryos were incubated for 30–40 min at room temperature, under gas, to allow for antibody binding. The embryos were washed three times in WB, and immediately cultured in cross-linking solution, as described below.

Embryo culture

Light mineral oil (5 ml; Fisher, Pittsburgh, PA, USA) was added to a capped 15 ml conical test tube containing 8 ml of WB without bovine serum albumin (BSA; Sigma) to allow for equilibration before the experiment began. The tube was shaken vigorously for several minutes to mix the oil and medium and then placed in the 37°C, 7% CO₂ incubator for the duration of the experiment (~3 h). At the

conclusion of the embryo collection, microdrops of culture medium were set up in a 35×10 mm culture dish (Corning; Corning, NY, USA). Then 20 µl of cross-linking solution [50 µg/ml of rabbit anti-mouse IgG (ICN, Aurora, OH, USA) and 5 ng/ml of PMA (Sigma) in WB medium] were placed on the bottom of the culture dish. The drops were covered by 3 ml of the equilibrated oil, and 10–12 embryos were transferred to each microdrop. The culture dish was covered and incubated at 37°C, 7% CO₂ for either 48 h (2-cell embryos) or 24 h (8-cell embryos) total time, at which point they were harvested.

Embryo harvesting

At 6 h before harvesting, embryos were removed from the incubator and the percentage in each microdrop reaching the blastocyst stage was assessed (a blastocyst was defined as an embryo with a blastocoel cavity). All embryos were removed from the microdrops and washed three times in WB. The embryos were then pipetted into wells of a 96-well plate (Becton Dickinson) containing 100 µl of WB. Next, 100 µl of a 8 µCi/ml solution of [³H-TdR] in WB were added to each well and the plate was covered and incubated for an additional 6 h at 37°C, 7% CO₂. After the final incubation period, embryos were harvested as described for T-cells, except that a separate Titertek harvester was used to eliminate background from the high levels of isotope in the T-cell experiments.

In some cases, 2-cell and 8-cell embryos were not incubated in radioisotope, but were kept in culture for longer periods of time. For these experiments, embryos were assessed for blastocyst formation at various time points. In addition, 8-cell embryos that had been cultured for 48 h were also assessed for hatching (when the embryo emerges from the zona pellucida in preparation for implantation). To quantify the number of cells per embryo, embryos were incubated in 200 µl of WB containing 5 µg/ml of bisbenzamide (Sigma), a nuclear staining dye, for 30 min at 37°C. The embryos were washed twice in WB, mounted in 10 µl of WB on a microscope slide, and the number of nuclei per embryo was counted at ×200 under UV light with an Olympus microscope.

Statistical analysis

Statistical analyses comparing treated embryos to control embryos were performed using the analysis of variance (ANOVA) program on Microsoft Excel.

Results

T-cell activation

In the first set of experiments, cross-linking reagents were tested for capability to induce Qa-2 mediated T-cell activation, using [³H-TdR] incorporation as an indicator of DNA synthesis. We incubated C57BL/6 T-cell enriched splenocytes with anti-Qa-2 mAb 1-12-1 or with control mAb 27-11-13, and then cross-linked the immune complexes with a secondary antibody in the presence of PMA. Figure 1 shows that cross-linking of Qa-2 induced a strong proliferative response from the T-cells. In a parallel set of experiments we tested our experimental system by stimulating T-cell activation with phyohaemagglutinin (PHA; data not shown). For comparison to Figure 1, treatment of T-cells with PHA resulted in 40×10³ c.p.m. incorporated, compared with 170×10³ c.p.m. incorporated after cross-linking with 50 µg/ml 1-12-1 mAb.

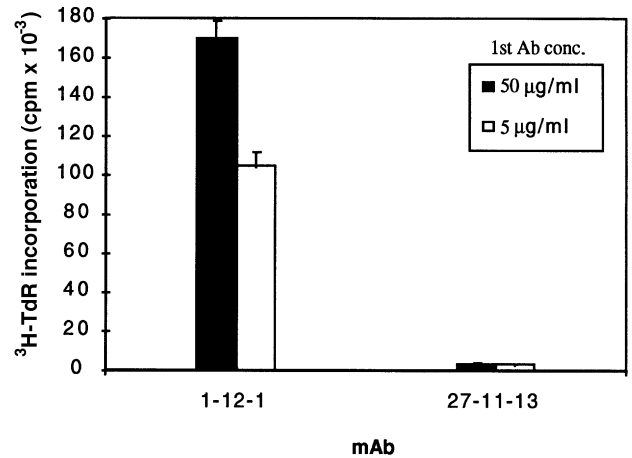


Figure 1. Activation of C57BL/6 T-cells after cross-linking of Qa-2 protein with secondary antibody, in the presence of 4/5-phorbol-12-myristate-13-acetate (PMA). T-cells (5×10⁵ cells/well) were incubated with anti-Qa-2 first antibody (1-12-1) or control first antibody (27-11-13), then with anti-mouse immunoglobulin G (IgG) second antibody and PMA for 48 h, as described in the text. One µCi of [³H-TdR] was added to each well for the last 6 h of culture and the cells were harvested. Results are from one representative experiment, performed in triplicate, and are presented as mean c.p.m. ± SEM.

Table I. Morphological assessment of 2-cell embryos from Qa-2 positive, *Ped fast* (C57BL/6) and Qa-2 negative, *Ped slow* (B6.K1) mice after treatment with anti-Qa-2 mAb (1-12-1) or control monoclonal antibody (mAb; TIB109) followed by a 72 h incubation in cross-linking solution.

Strain	No. of embryos	Mean percentage of blastocysts ± SEM	
		Control mAb	Anti-Qa-2 mAb
C57BL/6	251	63.4 ± 10.4	77.1 ± 6.7*
B6.K1	105	40.4 ± 22.1	42.3 ± 20.1

*Significantly different from control embryos of the same strain (P < 0.05).

Cross-linking studies on 2-cell preimplantation embryos

Initially, 1-12-1 mAb was incubated at different concentrations with C57BL/6 (Qa-2 positive, *Ped fast*) and B6.K1 (Qa-2 negative, *Ped slow*) 2-cell embryos and the percentage of embryos reaching the blastocyst stage assessed. Incubating 2-cell embryos in 0.5 mg/ml of 1-12-1 mAb, followed by a 48 or 72 h incubation in cross-linking solution (50 µg/ml of anti-mouse IgG+5 ng/ml PMA), were found to be the optimal conditions for increasing their cleavage rate (data not shown). These optimal conditions were used throughout the remainder of the embryo studies.

We found that C57BL/6 embryos treated with 1-12-1 mAb exhibited a significant increase (P < 0.05) in the percentage of blastocysts formed compared with embryos treated with control mAb, after 72 h in culture (Table I). As expected, the Qa-2- B6.K1 embryos did not exhibit a significant increase in cleavage rate.

We next quantified the incorporation of [³H-TdR] by C57BL/6 2-cell embryos 48 h after cross-linking of Qa-2, in the presence of PMA, as an indicator of the number of cells per embryo (Cozad *et al.*, 1981; Warner *et al.*, 1993b). C57BL/

6 embryos treated with 0.5 mg/ml of anti-Qa-2 mAb (1-12-1) had significantly higher ($P < 0.05$) incorporation of [³H-TdR] (i.e. number of cells per embryo) compared with embryos incubated in control mAb (TIB109) (Table II).

Cross-linking of Qa-2 on 8-cell embryos

The results from cross-linking Qa-2 protein on 2-cell embryos prompted us to continue the study on C57BL/6 and B6.K1 8-cell embryos. The 8-cell embryos were treated with cross-linking reagents and cultured for 48 h, when *Ped fast* embryos normally are expanded blastocysts and beginning to hatch from the zona pellucida. First, we evaluated the embryos for the percentage hatching from the zona pellucida (Table III). C57BL/6 8-cell embryos cultured for 48 h after cross-linking of Qa-2 protein exhibited a significant increase ($P < 0.05$) in the percentage hatching from the zona pellucida compared with control embryos. Second, we stained the nuclei in the embryos to quantify this effect, and found that C57BL/6 embryos treated with anti-Qa-2 mAb (1-12-1) had a significant increase ($P < 0.05$) in the total number of cells per embryo compared with embryos treated with control mAb (TIB109) (Table III). As expected, we were unable to detect an increase in cleavage rate in B6.K1 embryos treated with anti-Qa-2 mAb, compared with control mAb, as assessed by the number of cells per embryo and by the percentage hatching (Table III).

Discussion

The present study was undertaken to analyse the molecular mechanism and function of Qa-2 protein, the *Ped* gene product, in early mouse embryo development. Qa-2, a Class Ib MHC protein, is attached to the embryonic cell surface by a GPI linkage (Tian *et al.*, 1992). There are >100 known GPI-anchored proteins, which are structurally and functionally diverse (reviewed in Brown and Waneck, 1992). Several GPI-

linked proteins, including Qa-2, have been shown to be involved in signal transduction (reviewed in Brown and Waneck, 1992; Pratt and Gaulton, 1993; Horejsi *et al.*, 1999). Cross-linking of various GPI-linked proteins, in the presence of a co-stimulatory signal, causes T-cell activation and, therefore, it has been proposed that these proteins may serve an accessory role in the regulation of the immune response (Hahn and Soloski, 1989). We confirmed that cross-linking of Qa-2 protein induces T-cell activation, in agreement with results from several previous studies (Hahn and Soloski, 1989; Robinson *et al.*, 1989; Cook *et al.*, 1992; Hahn *et al.*, 1992).

We used the T-cell activation model to determine whether preimplantation embryos from the C57BL/6 *Ped fast* mouse strain could increase their rate of cleavage after Qa-2 cross-linking. We assessed the cleavage rate of preimplantation embryos by four criteria: the percentage of blastocysts formed, the percentage of embryos hatching from the zona pellucida, the amount of [³H-TdR] incorporation into DNA, and the total number of cells per embryo. Treatment of C57BL/6 2-cell embryos with anti-Qa-2 mAb followed by cross-linking with a second antibody, in the presence of PMA, resulted in a significant increase in cleavage rate compared to control embryos (Tables I and II). C57BL/6 8-cell embryos also increased their rate of cleavage after cross-linking of Qa-2 and culture for 48 h (Table III). Our results imply that the GPI-linked Qa-2 protein acts as a signal transduction molecule in response to cross-linking in preimplantation embryos.

It is not known if cross-linking of Qa-2 protein on the surface of C57BL/6 embryos would lead to an increased developmental rate or embryonic survival advantage *in vivo*. Cross-linking of GPI-linked proteins results in a highly specific primary signalling response that has been proposed to mimic the binding of natural ligands or receptors (reviewed in Robinson, 1997; Horejsi *et al.*, 1999). Two types of molecules are known that recognize MHC Class I molecules: the T-cell receptor and receptors on NK cells (reviewed in Le Bouteiller and Lenfant, 1996; Lanier, 1998). There may be other molecules, on the embryonic cells themselves, on cells in the uterine wall, or soluble molecules in the oviductal and/or uterine fluid, that recognize Qa-2 and mediate cross-linking in the early embryo. It is well known that embryos cultured *in vitro* cleave at a slower rate than embryos that develop *in vivo* (Streffer *et al.*, 1980; Brownell and Warner, 1988). However, co-culture of embryos in uterine washings or with uterine epithelial cells increases the in-vitro development rate (Sakkas *et al.*, 1989; Carney *et al.*, 1990; Fischer *et al.*, 1990),

Table II. Analysis of [³H-TdR] incorporation in 2-cell embryos from Qa-2 positive, *Ped fast* (C57BL/6) mice after treatment with anti-Qa-2 mAb (1-12-1) or control mAb (TIB109) followed by a 48 h incubation in cross-linking solution

Strain	No. of embryos	Mean c.p.m./embryo ± SEM	
		Control mAb	Anti-Qa-2 mAb
C57BL/6	195	165.0 ± 47.0	231.7 ± 48.1*

*Significantly different from control embryos of the same strain ($P < 0.05$).

Table III. Analysis of percentage of blastocysts hatching from the zona pellucida and quantitation of the number of cells per embryo in 8-cell embryos from Qa-2 positive, *Ped fast* (C57BL/6) and Qa-2 negative, *Ped slow* (B6.K1) mice after treatment with anti-Qa-2 monoclonal antibody (mAb; 1-12-1) or control mAb (TIB109) followed by a 48 h incubation in cross-linking solution

Strain	No. of embryos	Mean percentage of embryos hatching ± SEM		Mean number of cells per embryo ± SEM	
		Control mAb	Anti-Qa-2 mAb	Control mAb	Anti-Qa-2 mAb
C57BL/6	82	0.0 ± 0.0	7.3 ± 3.7*	26.3 ± 6.0	30.5 ± 5.9*
B6.K1	134	0.0 ± 0.0	1.4 ± 2.4	18.9 ± 5.7	18.1 ± 5.8

*Significantly different from control embryos from the same strain ($P < 0.05$).

suggesting that components in the maternal environment affect the embryonic cell cycle, some of which may signal through Qa-2. Additionally, since the *Ped* phenotype is maintained *in vitro*, potential ligand(s) or receptor(s) for Qa-2 may be of autocrine or paracrine origin.

Another factor that merits discussion is that cellular activation via cross-linking of GPI-linked proteins requires a co-stimulatory signal, which seems to influence the magnitude, not the nature, of the primary signal initiated by mAb binding (reviewed in Robinson, 1997). The embryos in this study were cultured in PMA, which causes premature compaction (Winkel *et al.*, 1990), cavitation (Ohsugi and Yamamura, 1993), and cell differentiation (Sawicki and Mystkowska, 1981) in preimplantation embryos. Additionally, following transfer to pseudopregnant females, PMA-treated embryos die shortly after implantation (Ohsugi and Yamamura, 1993). PMA activates protein kinase C (PKC), an enzyme whose activity is crucial for compaction and subsequent development (Winkel *et al.*, 1990). Any treatment that perturbs the activity of PKC, and/or that of any other cell cycle molecule, may disturb the intrinsic embryonic cell clock (Ohsugi and Yamamura, 1993). Since the embryos in this study were co-cultured in PMA, it would be interesting to cross-link Qa-2 on the surface of C57BL/6 embryos and transfer the blastocysts to pseudopregnant mothers to determine their survival rate *in vivo*.

Precisely how Qa-2 mediates cell signalling in T-cells and in preimplantation embryos is not yet known, although basic models have been proposed (reviewed in Malek *et al.*, 1994). One model proposes that the GPI-molecule undergoes endocytosis after ligand binding. The endocytosis of certain GPI-linked proteins is associated with the actin cytoskeleton, and is tightly regulated by protein kinases C and A (Deckert *et al.*, 1996). From T-cell studies using soluble and immobilized mAbs, Thy-1 and Ly-6 endocytosis has been shown to be required for activation following cross-linking (Bamezai *et al.*, 1989), but Qa-2 endocytosis is not required for its role in activation (Cook *et al.*, 1992). We do not yet know if internalization of Qa-2 occurs in or is required for signal transduction in embryos, or if Qa-2 is associated with the cytoskeleton in embryos.

The widely favoured model of how GPI-linked proteins signal is that GPI-linked proteins associate with other proteins. Immunoprecipitation studies have shown that various GPI-anchored proteins associate with src-family member tyrosine kinases (Stefanova *et al.*, 1991; Shenoy-Scaria *et al.*, 1992, 1993; Draberova *et al.*, 1996), heterotrimeric G protein α subunits (Solomon *et al.*, 1996), integrins (Sammar *et al.*, 1997), and transmembrane receptor kinases (Buj-Bello *et al.*, 1997; Klein *et al.*, 1997). The way in which these molecules associate may be functionally relevant. The proteins and associated lipids appear to cluster in membrane 'microdomains' that act as hotspots for signal transduction (reviewed in Brown, 1992; Brown and Waneck, 1993; Horejsi *et al.*, 1999; Jacobson and Dietrich, 1999). Upon cross-linking or ligand binding, the proteins and lipids are probably activated by being brought in closer proximity to each other through dimerization or oligomerization, which are frequently used strategies for trans-

ducing signals from the cell surface to the nucleus (reviewed in Klemm *et al.*, 1998).

Identification of potential accessory molecules that recognize Qa-2 may be critical for determining its mode of action. In lymphocytes, a 110 kDa transmembrane protein associated with Qa-2 was phosphorylated after anti-Qa-2 mAb binding (Robinson and Hederer, 1994). Additionally, the insulin receptor associates with Class I MHC molecules and can bind Class I MHC molecules in place of $\beta 2$ microglobulin (Due *et al.*, 1986). Insulin receptors are expressed in preimplantation embryos and physiological concentrations of insulin stimulate cleavage rate (Rao *et al.*, 1990). It is tempting to speculate that these or other proteins may associate with the Qa-2 protein in *Ped fast* preimplantation embryos, acting as mediators in the signal transduction pathway that leads to a rapid rate of cleavage.

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