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Porin isoform 2 has a different localization in *Drosophila melanogaster* ovaries than porin 1

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Abstract Eukaryotic porins or VDACs are a class of transmembrane proteins mainly localized in the outer mitochondrial membrane, whose function is to allow the diffusion of metabolites between the cytosol and the mitochondrion. In *Drosophila melanogaster*, as in other organisms, a small family of genes encoding porins has been discovered from the sequence of the genome. It is of general interest to understand whether these genes represent functional entities or not, and whether their product is associated to any particular tissue. In previous work we reported about the transcriptional and translational analysis of *porin 1* and *porin 2*, and we proposed the specific presence of the latter in spermatozoa of the fly. In this paper we performed real time RT-PCR quantification of *porin 1* and *porin 2* transcripts in germ cells of the fly. It indicates that Porin 1 is abundantly expressed in both male and female tissues; Porin 2 instead, is very abundant in testis and it is present in ovaries as well, but in a small amount. The immuno-histological stain of ovaries shows that Porin isoform 1 is selectively targeted to follicular cells while Porin isoform 2 is present in mitochondria of the epithelial sheath cells of the ovariole. The implications of the subcellular distribution of these porin isoforms and the specific localization in germ tissues are discussed.

Keywords Voltage dependent anion selective channel · Porin · Mitochondria · *Drosophila melanogaster* · Ovary · Ovariole · Follicular cells · Real time RT-PCR

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Introduction

Drosophila melanogaster has a cluster of genes encoding the mitochondrial porins or VDACs (voltage-dependent anion-selective channels) localized in the small region 32B3-4 on the chromosome 2L (Oliva et al. 2002; De Pinto et al. 2003). These genes are probably derived by duplication events during evolution. As in other cases, only the leader gene (*porin 1* or *CG6647*) has been associated to a protein by the classical tools of biochemistry and genetics (De Pinto et al. 1989; Messina et al. 1996; Oliva et al. 1998). The additional genes (*porin 2*, *3* and *4*, also called from their gene nomenclature *CG17137*, *CG17139* and *CG17140*) have been discovered and localized by the extensive genome sequencing efforts. It is of general interest to understand whether these genes represent functional entities or not, and whether their function is associated to any particular tissues or, as in the case of the insects, to any specific stage in the development of the organism. Another reason of interest is linked to the function of the VDACs or porins. They are a group of transmembrane pore-forming proteins predicted to be β-barrel structures with connecting

loops (Casadio et al. 2002; Young et al. 2007). This kind of organization is, as far as it is known, specific for the mitochondrial outer membrane proteins (Rapaport 2003). Sequence-based structural predictions showed that all the putative porin genes in *D. melanogaster* should contain β-barrels (Casadio et al. 2002; Rapaport 2003; Young et al. 2007). Eukaryotic porins have been demonstrated to regulate the exchange of ATP/ADP through the outer mitochondrial membrane (Rostovtseva and Colombini. 1997). For this crucial position in the cell as key regulator of the bioenergetic metabolism, VDAC has been called the "governator" of the cell (Lemasters and Holmuhamedov 2006). VDAC has also been implicated in apoptosis (for a review see Kroemer et al. 2007), since it could contribute to the permeabilization events that lead to the activation of caspases by intra-mitochondrial proteins.

Little is known about the function of VDAC or porin isoforms discovered after the most abundant Porin 1. To gain information about the cellular existence and the physiological context of the Porin 2 (i.e. the product of gene *porin 2* or *CG17137* in *D. melanogaster*), we produced antibodies against the Porin 2 protein expressed in vitro and performed a number of functional experiments and transcriptional and translational analyses (Aiello et al. 2004; Guarino et al. 2006). Porin 2 is expressed in *Drosophila melanogaster* by a conserved gene. A recent report compared the sequence of 12 genomes of the *Drosophila* genus (Drosophila Genome Consortium 2007). The gene encoding Porin 2 is present in all of them and has a conserved structure (three exons: the only exception is *D. willingstoni* where the gene was split in four exons) (Drosophila Genome Consortium 2007). The protein sequence is also pretty well conserved and specific residues, found to be functionally important like E66 (Aiello et al. 2004), were found conserved in 11/12 sequences. This observation indicates that *porin 2* is a conserved, useful gene for the fly.

Porin 2 is expressed in embryos, pupae and adults but much less at the larval stage (Aiello et al. 2004). In a previous work we reported that RT-PCR amplifications corresponding to the *porin 2* coding sequences were detected both in testis and in ovaries. Nevertheless in ovaries the amplification with primers corresponding to the start and stop codons produced an amplicon that, upon sequencing, contained the two internal introns present in the gene. Amplifications performed using the testis polyA+RNA as a template originated the expectedly spliced sequences (Guarino et al. 2006).

In a new effort to study the expression products of this gene we prepared polyA+RNA from ovaries by means of another procedure and we analyzed the transcripts by a real time RT-PCR protocol. With the real time RT-PCR we did not find any trace of an immature transcript. We are thus driven to the conclusion that the result obtained in our previous work (Guarino et al. 2006) was caused by an incomplete degradation of the contaminating genomic DNA. In this conclusion we are supported by (Graham and Craigen 2005). In this paper we present the complete quantitative analysis of the *porin 1* and *2* transcripts in the fly germ tissues. The porin-protein presence in ovaries was investigated by immuno-fluorescence microscopy leading to stunning new information. A specific, different localization with developmental implications has been assigned to both porin isoforms.

Experimental procedures

Western blot analysis of *D. melanogaster* tissues

Protein samples were prepared from whole bodies and from dissected tissues of wild type flies by squashing them and extracting the proteins in sample buffer. Samples were electrophoresed on 0.75 mm slab gels, electroblotted onto nitrocellulose membrane (Amersham) and probed with antisera at the indicated dilutions. SDS-PAGE and immunoblotting were carried out with standard protocols (Guarino et al. 2006). Antibody reactivity was visualized with ECL (Amersham).

Preparation of mitochondria from ovaries

About 600 ovaries from adult flies (*Oregon-R)* were prepared by dissection and homogenated with potter in 1 ml of Buffer A containing 5 mM HEPES, pH 7.2; 210 mM mannitol, 70 mM sucrose, 1 mM EGTA. The homogenate was spun down at 150×*g* for 4 min. The supernatant was collected and re-centrifuged at 9,000×*g* for 10 min. This supernatant was centrifuged once more at 9,000×*g* for 10 min. The two pellets were collected in the minimum volume of Buffer A and represent the mitochondrially enriched fraction (P) used in Western blots.

Quantitative real-time RT-PCR

Total RNA was extracted from 30 mg of male and female germinal tissues using RNAqueos-4 PCR Kit (AMBION) reagent following manufacturer's protocol. To remove any DNA from the preparation, the samples were incubated with DNase I RNase free (AMBION) (1 U/mg RNA) at 37 °C for 15 min, in a total volume of 100 μl. After this treatment, the enzyme was inactivated with the DNase inactivation reagent (AMBION). DNase treated RNA was precipitated at −80 °C overnight and after centrifugation it was dissolved in 30 μl of distilled water. The RNA concentration and purity were determined photometrically by measuring its absorbance at 260 nm and the A_{260}/A_{280} ratio. In the first-strand cDNA synthesis, 5 μg of

total RNA were used as a template for oligonucleotides dT primed reverse transcription using SuperScript III RNase H-reverse transcriptase (Invitrogen), according to manufacturer's instructions.

Real Time RT-PCR was performed in the SmartCycler Real-time PCR (Cepheid). Relative abundance of the *porin1* and *porin2* transcripts in wild type germinal tissues was determined using Fluo Cycle™ for SYBR Green (Celbio) according to manufacturer's protocol. For quantification of the transcripts we used the standard curve method as described in (Ponhcel et al. 2003; Larionov et al. 2005). The standard curve was obtained with the Dm*porin1* cDNA and the pQE30*porin2* cDNA (Aiello et al. 2004) and with the *rp49* cDNA. Real time RT-PCR was performed with the following primers' couples: *por1* upper 5′ GAAA CAAGAACGGCAAGT 3′ and *por1* lower 5′ TGTGTGT CAAATGCGGTC 3′; *por2* upper 5′ CTGACCCTAACC GAGCGA 3′ and *por2* lower 5′ CCACAACCAGCGAA CAGT 3′; *rp49* upper 5′ ATCGGTTACGGATCGAACAA 3′ and *rp49* lower 5′ GACAATCTCCTTGCGCTTCT 3′. PCR conditions were: 10 min 95 °C; 30 s 95 °C; 30 s 53 °C (*porin1*) or 30 s 56 °C (*rp49* and *porin2*); 30 s 72 °C. The last three steps were repeated for 45 times.

We performed the experiments in triplicate (with three independent cDNA preparations) and we calculated the mean value and the standard deviations.

In the standard curve method, sample amount was calculated as follows: sample [pg]=$10^{(ct\ sample\ -\ intercept)/slope}$. Normalization for each determination was obtained by dividing each quantitative value calculated by the quantitative value of the *rp49* transcript, in experimental samples.

Immunofluorescence microscopy

Ovaries were dissected in PBS (phosphate-buffered saline: 130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, pH 7.4), and hybridization was carried out as described in (Lehmann and Tautz 1994). The fixed material was then incubated overnight with the primary antibody. The following primary polyclonal antisera were used: a mouse anti-Porin 1 and anti-Porin 2 (dilution 1:100) (Guarino et al. 2006), and a rabbit anti-ATP synthase β subunit (1:200) [a gift from Dr. Rafael Garesse]. The staining was detected with the following secondary antibodies: a fluorescein-isothiocyanate (FITC)-labelled goat anti-mouse IgG (LiStarFish) and a tetramethylrodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit IgG (from Molecular Probes) (dilution 1:500). Nuclear DNA was stained with DAPI. Digital images were obtained using a Nikon Optiphot-2 fluorescence microscope equipped with a CCD camera. Specific gray scale images from FITC and DAPI fluorescence were computer colored and merged using the Adobe Photoshop software.

Results

Real time RT-PCR shows the presence of the mature *porin 2* transcript in *D. melanogaster* ovaries

Real Time RT-PCR experiments allowed us to refine our analysis of transcripts in the fly germ tissues. Upon amplification of a new polyA+RNA preparation obtained from testis and ovaries we calculated the amounts of *porin 1*, *porin 2* and *rp49* transcripts for each single determination and the relative amounts of *porin 1* and *porin 2* transcripts with respect to the *rp49* transcript (as an internal control) in triplicate. We also calculated the mean value and the standard deviation (Table 1). Our results show that, in male germinal tissues, both *porin 1* and *porin 2* transcripts are expressed in ovaries at comparable, high levels (about 57% and 42% with respect to the *rp49* transcript). Instead the *porin 1* and *porin 2* transcripts are expressed at different rates: *porin 1* transcript is very abundant, since it is about 14 folds than the *rp49* transcript; on the other hand *porin 2* transcript is expressed at a very low rate, in fact it represents only 1% of the *rp49* transcript.

Western blot analysis of *D. melanogaster* female germinal tissues

To verify the presence of the two Porin proteins in the ovary we performed Western blots of male and female germinal tissue extracts that confirmed the results of the real time RT-PCR. Porin 1 is abundantly expressed in testis, ovaries and extracts from the remaining part of the body (Fig. 1a). The same experiment performed with anti-Porin 2 antibodies revealed that Porin 2 was sufficiently expressed in testes' extracts while only faint bands were visible in the ovaries and in the remaining part of the body (Fig. 1a).

Mitochondria were purified from ovaries to detect the presence of the two Porin isoforms in the organelle. Both anti-Porin1 and anti-Porin2 sera stained the respective protein in the ovaries' mitochondria in Western blots experiments (Fig. 1b–c). It is noteworthy that Porin 2 was detected with a very low dilution of the antisera, in comparison to Porin 1, indicating a lower concentration of the protein. This result is in agreement with the minor abundance of *porin 2* mRNA detected in real-time PCR.

Histological staining of the *D. melanogaster* ovaries with anti-Porin 1 and anti-Porin 2 Abs reveals a different tissue localization of these isoforms

The histological staining of the *D. melanogaster* ovaries was thus attempted with anti-Porin 2 antibodies and, for comparison, with anti-Porin 1 antibodies. The application of the antiserum raised against recombinant Porin 2 towards

Table 1 Threshold cycles (Ct), concentration of *porin 1, porin 2* and *rp49* transcripts and the corresponding relative amount of transcripts, expressed in picograms, in three different cDNAs extracted from wild type flies

	Ct *rp49*	Ct *porin1*	Ct *porin2*	*rp49* in pg	*porin1* in pg	*porin2* in pg	*porin1/rp49*	*porin2/rp49*
Porin 1 and 2 in *Oregon-R* testes								
Porin 1								
Sample 1	19.55	27.53		34.56	18.68		0.54	
Sample 2	19.35	27.28		39.48	22.00		0.56	
Sample 3	19.8	27.56		29.24	18.31		0.63	
Mean value							0.57	
Standard deviation							0.04	
Porin 2								
Sample 1	19.55		20.81	34.56		13.89		0.40
Sample 2	19.35		20.67	39.48		15.21		0.39
Sample 3	19.8		20.86	29.24		13.45		0.46
Mean value								0.42
Standard deviation								0.04
Porin 1 and 2 in *Oregon-R* ovaries								
Porin1								
Sample 1	21.8	24.5		7.70	135		17.54	
Sample 2	21.6	25.78		8.80	59		6.71	
Sample 3	22.0	24.7		6.74	118		17.52	
Mean value							13.92	
Standard deviation							6.24	
Porin 2								
Sample 1	21.8		32.2	7.70		0.09		0.012
Sample 2	21.6		34.2	8.80		0.09		0.010
Sample 3	22.0		34.11	6.74		0.03		0.004
Mean value								0.01
Standard deviation								0.004

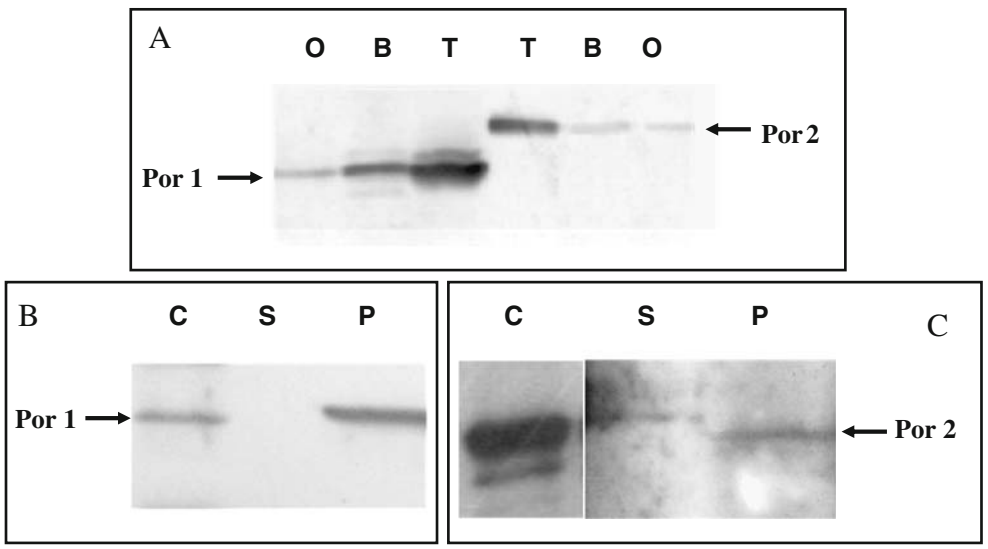


Fig. 1 Molecular analysis of Porin 1 and Porin 2 in germinal tissues. **a** Western blot of male and female *D. melanogaster* tissue extracts by antisera anti-Porin 1 and anti-Porin 2. Ovaries (*O*), whole bodies (*B*) and testis (*T*) extracts of adult flies were analysed by Western blot. The *left panel* shows the staining by anti-Porin 1, the right panel by anti-Porin 2. The primary antisera were diluted 1:1,000 (anti-Porin 1) and 1:100 (anti-Porin 2). **b** Western blot of mitochondria prepared from *D. melanogaster* ovaries with antisera anti-Porin1. A fraction enriched in mitochondria was obtained by ovaries dissected form adult flies. The fraction enriched in mitochondria or mitochondrial pellet (*P*), the supernatant (*S*) and the recombinant Porin 1 as a control (*C*) were blotted and immunostained with anti-Porin 1 (1:1,000). **c** Western blot of mitochondria prepared from *D. melanogaster* ovaries with antisera anti-Porin 2. The same fractions described in **b** were immunostained with anti-Porin 2 (1:100). *P* Mitochondrial pellet, *S* supernatant, *C* recombinant Porin 2 as a control

fresh ovaries from *D. melanogaster* resulted in the unexpected stain of a histological discrete structure (Fig. 2h,i,k,l). The immunofluorescence stain obtained with the antiserum anti-Porin 1 showed that also Porin 1 is present in ovaries, but in a different district (Fig. 2b,c,e,f). Both stains were very selective. The anti-Porin 1 antibody detects exclusively ovary follicular cells (Fig. 2a–f). The anti-Porin 2 antibody stains the envelope of the ovariole, the structure where each single egg cell reaches maturity (Fig. 2g–l). This envelope is mainly formed by muscle cells. We identified this structure as the "epithelial sheath", comparing the anti-Porin 2 immunostaining with the scheme of the epithelial sheath reported in Hudson et al. (2008). No staining at all was detected in the internal cells and in the oocyte, which were stained only by the DNA-specific DAPI (Fig. 2a,d,g,j).

To clarify the intracellular localization of Porin 2 in the epithelial sheath cells, we performed a histological co-staining of the *D. melanogaster* ovaries with anti-Porin 2 and anti-ATP synthase antibodies. ATP-synthase β-subunit is a mitochondrial protein, thus it was used as a marker of the organelle membrane, as reported both in germline and in somatic cells (Cox and Spradling 2003). In our experiments it was shown that the specific staining of Porin 2 has, indeed, a peculiar distribution (Fig. 2m). In fact it is distributed along the external border of the cell membrane. In this distal localization there is only a partial overlap with ATP synthase that is concentrated slightly more inside the cell, as demonstrated by the partial merging of the separate staining. Unfortunately it is not really possible to state whether such merging is due to the enlargement of the intense ATP synthase staining, or to a molecular proximity of the two antigenes in vivo.

Discussion

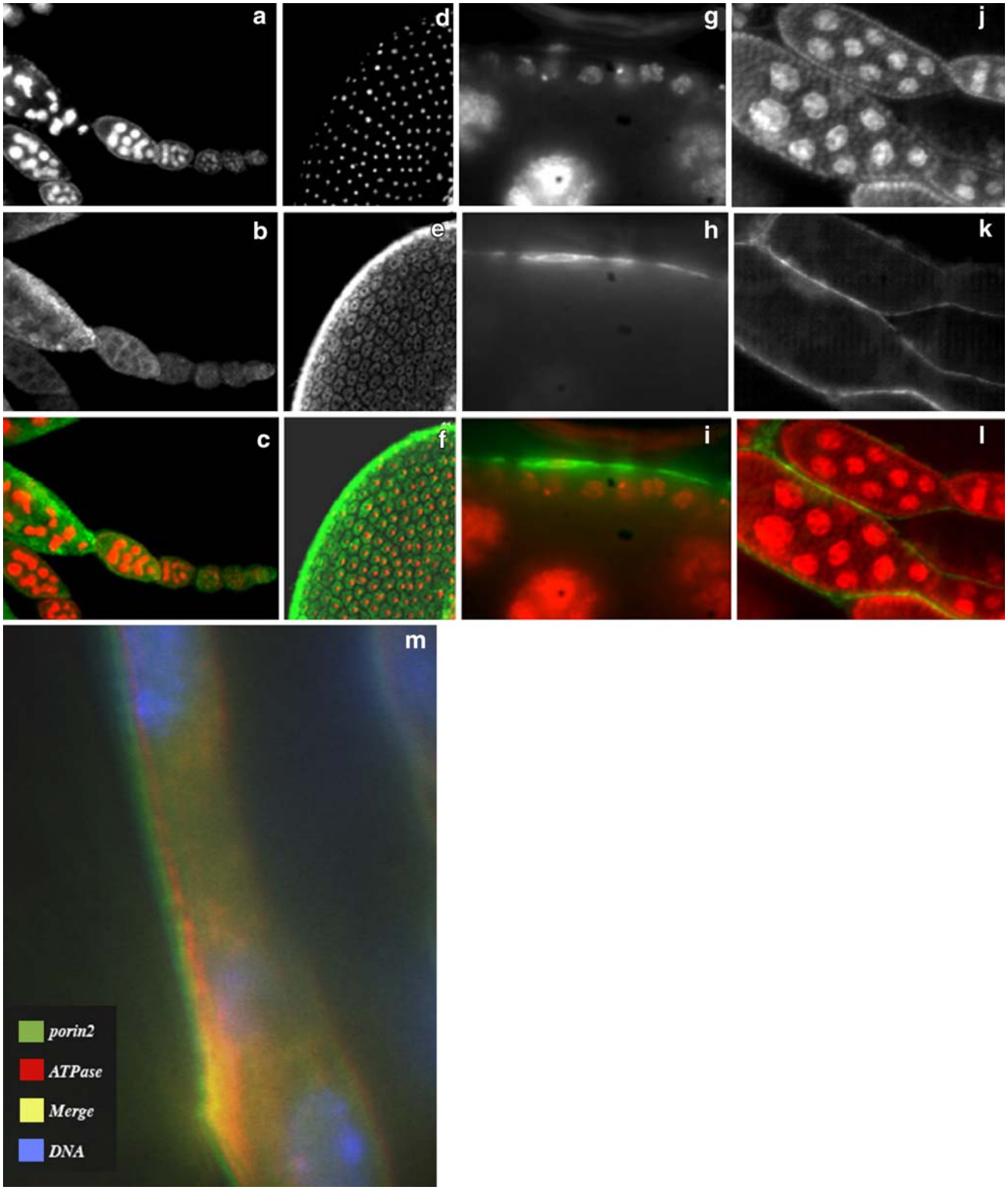
We reported in two papers about the expression of the *porin 2* gene in *D. melanogaster*. In the present work we have shown that *porin 1* and *porin 2* in *Drosophila melanogaster* germ cells have different transcriptional levels and specific protein distribution in the tissues.

Real time RT-PCR quantification of *porin 1* and *porin 2* transcripts in germ cells of the fly indeed indicates that *porin 1* is abundantly present in both male and female tissues, while *porin 2* is present in higher amounts in testes than in ovaries. The results at a protein level confirm this asymmetrical level of expression, showing that both male and female germ tissues are rich in Porin 1. Porin 1 is specifically localized in mitochondria as seen in testes (Oliva et al. 2002) and here in ovaries. Similar Western blot experiments detected Porin 2 in testes (Guarino et al. 2006) and here in ovaries at low levels, relative to Porin 1, confirming the real time RT-PCR analysis. Interestingly in ovaries both Porin isoforms were found in a mitochondrially enriched fraction.

Histochemical staining with monospecific polyclonal antibodies was performed. Anti-Porin 1 and anti-Porin 2 antibodies produced in our laboratory and an anti-ATP synthase β-subunit antibody were used to detect the distribution of the pore-forming proteins. Both anti-porin antibodies were able to show very specific and peculiar fluorescence stainings and the anti-ATP synthase β-subunit was useful to confirm the localization of mitochondria since it was found to label mitochondria in both germline and somatic cells of the ovary (Cox and Spradling 2003).

Specific and differentiated localization of Porin 2 in *D. melanogaster* ovaries

The *porin 2* mRNA is able to drive the synthesis of a protein localized very specifically in the ovary. Our antibodies detected with high specificity the "epithelial sheath" of the ovariole. This structure is a layer of thin circular muscles surrounding each of the 16 ovarioles. Recent studies on the *Drosophila* ovarian muscle morphology and sarcomere organization have characterized the muscle cells of the epithelial sheath (Middleton et al, 2006; Hudson et al, 2008). This muscle derives from mesodermal cells in the apical pole of pupal ovaries (King 1970) and it is a striated muscle with a typical sarcomere (Hudson et al. 2008; King 1970). Each epithelial sheath cell contains a single nucleus, showing that they differentiate without fusing into multinuclear myotubes. The epithelial sheath cells are mesh-like in shape (Hudson et al. 2008). The localization of the Porin 2 into the ovarian epithelial sheath muscle cells is demonstrated by the specific staining of the anti-Porin 2 in a filamentous structure that corresponds to the cytoplasm of these particular cells. This cytoplasm closely surrounds each sarcomere with large gaps between sarcomeres connected by thin cytoplasmic bridges (Hudson et al. 2008). Co-immuno staining with a mitochondrial marker (the anti ATP synthase β subunit) gave a clear indication for a mitochondrial localization, as found in Western blots. On the other hand it seems that the localization of Porin 2 could not be exclusively mitochondrial, in fact the superimposition of the two stainings did not show a complete overlapping between the two proteins. From the images one could suppose that the two proteins are localized very close, perhaps in two membranes tightly parallel: it could be the case of the outer and inner membrane of mitochondria or of the mitochondria and the plasma membrane of the muscle cell since the mitochondria are distributed at the periphery of the muscle fibers. The localization of VDAC 1 in the sarcoplasmic reticulum has been already demonstrated in amphibian and mammalian skeletal muscles



(Junankar et al. 1995; Shoshan-Barmatz et al. 1996). In this context this protein has been proposed to control the Ca^{2+} exchange (Szabadkai et al. 2006). We have found that the Porin 2 is cation-specific instead of anion specific, as it is more common (Aiello et al. 2004). There is also evidence of the presence of VDAC in the plasma membrane of various other kinds of cells (for a review see Bathori et al. 2000) where it could have a redox activity,

◀ **Fig. 2** Immunolocalization of Porin isoforms 1 and 2 in female germ tissues of *Drosophila melanogaster*. Several ovaries from wild type (*Oregon-R*) females were incubated with an anti-Porin 1 antibody (**a**–**f**) or an anti-Porin 2 antibody (**g**–**l**). DNA in the nuclei was stained with DAPI (**a**, **d**, **g**, **j**). The primary antibodies were revealed with a FITC-labeled secondary antibody (**b**, **e**, **h**, **k**). Gray scale images separately obtained by specific filters were computer colored (DAPI *red* and FITC *green*) and merged to give the final image (**c**, **f**, **i**, **l**). Porin 1 is detected in the follicular cells surrounding the oocyte. In **a**–**c** an ovariole is shown. **d**–**f** A small region of the oocyte surface. Porin 2 is specifically found in the muscular envelope surrounding the ovariole (**h**, **k**). **m** Immuno-colocalization of Porin 2 and ATP-synthase β subunit in female germ tissues of *Drosophila melanogaster*. Ovaries from wild type (Oregon-R) females were incubated with a mouse antibody anti-Porin 2 and a rabbit antibody anti-ATP synthase. Gray scale images in the various channels were obtained separately by specific filters. DNA in the nuclei was stained with DAPI (in *blue* in the merged figure). The primary antibodies were revealed with an anti-mouse FITC-conjugate secondary antibody (in *green* in the merged figure) and an anti-rabbit TRITC-conjugate secondary antibody (in *red* in the merged figure) respectively. These images report a small region of the epithelial sheath. Magnification: **a**–**c**: ×20. **d**–**i**: ×100. **j**–**l**: ×40. **m**: ×100

capable of reducing extracellular ferricyanide in the presence of intracellular NADH (Baker et al. 2004).

Other reports concern a structural role of the porin proteins. For example it has been demonstrated that VDAC2 and VDAC3 are present in the Outer Dense Fibers of spermatozoa (Hinsch et al. 2004). The absence of at least one of them was associated with a defective organization of the axonema, provoking the sperm immotility (Sampson et al. 2001). Furthermore it has been described the interaction of mitochondrial VDAC with cytoskeletal proteins as MAP2 (microtubule associated protein 2) (Linden and Karlsson 1996).

In *Drosophila* males Porin 2 has been found enriched in the sperm flagellum (germ cell line) (Guarino et al. 2006). This means that (a) the same gene has to be differentially expressed in the developing reproductive apparatus in the two sexes; (b) the protein product might have different functions in the two sexes.

Porin 1 in *D. melanogaster* ovaries

For comparison, we analyzed the distribution of the Porin 1 (or VDAC) in ovaries. The antibodies anti-Porin 1 resulted in a very specific reactivity with structures in the follicular cells surrounding the oocyte. These should represent the mitochondria, as can be seen for comparison in similar images shown in Cox and Spradling (2003). Porin 1 is indeed expressed in follicular cells surrounding the oocyte, most likely in the mitochondria, since the staining is relatively diffuse in the whole cell with the exception of nuclei. On the other side, the finding that it has been localized in the mitochondria well correlates with a previous work in which the Porin 1 has been localized in the nebenkern body, precursor of mitochondria in the testis (Oliva et al. 2002).

More experiments are essential to specifically investigate the Porin 1 presence in the mitochondria of the Balbiani body in the oocyte. In our experiment the interpretation of the labeling pattern in the oocyte is really complicated by the interference with signals from underlying follicle cells.

Human VDAC1 and VDAC2 were identified in the human ovary (Wang et al. 2005); VDAC has been detected also in the oocytes of *X. laevis*. Steinacker et al. (2000) described the localization of endogenous VDAC in the *X. laevis* oocyte plasma membrane by immunofluorescence confocal microscopy.

Conclusions

The definitely clear, different tissutal distribution of the protein products of the two porin genes in *Drosophila* female germ cells raises interesting considerations and poses new questions. The differentiated expression patterns found in the ovaries of the fly ask for specific mechanisms able to control the gene activity in order to obtain the peculiar distribution reported in this work. This requires a subtle tuning of the two genes that are spatially very close on the chromosome. We have already shown that a certain degree of coordination in the expression of Porin 1 and Porin 2 genes exists (Guarino et al. 2006). On the other side, different mechanisms controlling the expression of the genes should take place to specify the presence of the different isoforms in different districts. The localization of isoforms in different districts has been already shown in various organisms and tissues. In mouse the differential targeting of two sodium channel isoforms in the same axon is a process associated with the formation of compact myelin during development (Boiko et al. 2001). In *Drosophila* the different expression and distribution of Shaker potassium channels has been shown during the development of the nervous system (Rosero et al. 1997). In mouse oocytes, inositol trisphosphate receptor isoforms present differential distribution (Fissore et al. 1999). This work undoubtedly demonstrates specific, differentiated localizations of at least two porin isoforms, Porin 1 and Porin 2, in ovaries. This finding suggests that *porin 1* and *porin 2* genes have evolved a different function and regulation.

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