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Relating proton pumps with gap junctions: colocalization of ductin, the channel-forming subunit c of V-ATPase, with subunit a and with innexins 2 and 3 during *Drosophila* oogenesis

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Abstract

Background: Ion-transport mechanisms and gap junctions are known to cooperate in creating bioelectric phenomena, like pH gradients, voltage gradients and ion fluxes within single cells, tissues, organs, and whole organisms. Such phenomena have been shown to play regulatory roles in a variety of developmental and regenerative processes. Using *Drosophila* oogenesis as a model system, we aim at characterizing in detail the mechanisms underlying bioelectric phenomena in order to reveal their regulatory functions. We, therefore, investigated the stage-specific distribution patterns of V-ATPase components in relation to gap-junction proteins.

Results: We analysed the localization of the V-ATPase components ductin (subunit c) and subunit a, and the gap-junction components innexins 2 and 3, especially in polar cells, border cells, stalk cells and centripetally migrating cells. These types of follicle cells had previously been shown to exhibit characteristic patterns of membrane channels as well as membrane potential and intracellular pH. Stage-specifically, ductin and subunit a were found either colocalized or separately enriched in different regions of soma and germ-line cells. While ductin was often more prominent in plasma membranes, subunit a was more prominent in cytoplasmic and nuclear vesicles. Particularly, ductin was enriched in polar cells, stalk cells, and nurse-cell membranes, whereas subunit a was enriched in the cytoplasm of border cells, columnar follicle cells and germ-line cells. Comparably, ductin and both innexins 2 and 3 were either colocalized or separately enriched in different cellular regions. While ductin often showed a continuous membrane distribution, the distribution of both innexins was mostly punctate. Particularly, ductin was enriched in polar cells and stalk cells, whereas innexin 2 was enriched in the oolemma, and innexin 3 in centripetally migrating follicle cells. In lateral follicle-cell membranes, the three proteins were found colocalized as well as separately concentrated in presumed gap-junction plaques.

Conclusions: Our results support the notion of a large variety of gap junctions existing in the *Drosophila* ovary. Moreover, since ductin is the channel-forming part of a proton pump and, like the innexins, is able to form junctional as well as non-junctional membrane channels, a plethora of cellular functions could be realized by using these proteins. The distribution and activity patterns of such membrane channels are expected to contribute to developmentally important bioelectric signals.

Keywords: *Drosophila melanogaster*, Oogenesis, Bioelectricity, Cell communication, Pattern formation, Proton pump, Gap junction

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Background

The concerted action of ion-transport mechanisms and gap junctions gives rise to bioelectric phenomena, like pH gradients, voltage gradients and ion fluxes within cells, tissues, organs, and even organisms. Such bioelectric phenomena are known to play regulatory roles in various developmental and regenerative processes, e.g. proliferation, differentiation, polarisation and migration [1–5].

Using a variety of approaches, we have gathered considerable information on the electrical properties as well as on the distribution and activity patterns of ion-transport mechanisms and gap junctions in the ovary of *Drosophila melanogaster* [6–19]. Following this strategy in a model system, we are trying to further clarify the roles that bioelectric phenomena play during development, e.g. for pH-regulation, osmoregulation, cell-cell communication, cell migration, cell proliferation, cell death, vitellogenesis and growth.

During the course of *Drosophila* oogenesis, characteristic extracellular current patterns [6, 20] as well as membrane-potential changes in germ-line and soma cells have been observed that partly depend on the exchange of protons, potassium ions and sodium ions [7, 21, 22]. Moreover, striking stage-specific patterns of membrane potentials and intracellular pH that characterize distinct cell populations have been described recently [19]. These bioelectric patterns were found to be related to the distribution patterns of various membrane channels, namely V-ATPases, L-type Ca^{2+} -channels, amiloride-sensitive Na^{+} -channels and $\text{Na}^{+},\text{H}^{+}$ -exchangers, as well as innexin-3-containing gap junctions. Therefore, these membrane proteins are likely to be involved in the regulation of membrane potentials and/or intracellular pH [19].

An ovarian follicle of *Drosophila* consists of 16 germ-line cells surrounded by a layer of somatic follicle cells [23, 24]. The oocyte and its 15 nurse cells form a cytoplasmic continuum via intercellular bridges as well as via gap junctions, and the same holds true for the follicle cells. On the other hand, the germ-line cells are connected to the soma cells only via gap junctions [25, 26]. By way of the intercellular exchange of microinjected fluorescent tracers, stage-specific communication between germ-line cells and soma cells has been detected, and a variety of treatments was found to either inhibit or stimulate this communication [12].

Innexins are considered the main gap-junction proteins of invertebrates [27–30], but there is some evidence that additional proteins, like e.g. ductin, can be part of gap junctions [11, 15, 31]. Therefore, it is tempting to analyse whether or not ductin and members of the innexin family are found in common gap-junction plaques. Previously, the localization of ductin [11, 16] as well as the localization of innexins 1 to 4 [18] have been separately analysed in *Drosophila* ovarian follicles in detail.

In the present study, we try to clarify the relationship of ductin, known as a channel-forming subunit of V-ATPases, with innexins 2 and 3, two well known components of gap junctions, using double-immunolabeling. We apply combinations of four antisera directed against (1) ductin (proteolipid or subunit c), (2) another V-ATPase component in the membrane-spanning V_0 domain (subunit a), (3) innexin 2, and (4) innexin 3, respectively. These antisera have previously been shown to specifically recognize cellular antigens in the *Drosophila* ovary [18, 19].

Methods

Preparation of follicles

Drosophila melanogaster wild-type Oregon R flies were reared at about 20 °C on standard food with additional fresh yeast. Individual 2–3 days old females were killed by crushing the thorax with tweezers without previous anesthetization. The ovaries were dissected with tweezers in *Drosophila* PBS [32], and single follicles of different stages were isolated by pulling at the anterior tip of an ovariole.

Antisera

To localize V-ATPase and gap-junction proteins, we used (1) a rabbit antiserum (Anti-ductin [19]) raised and affinity-purified against a highly conserved region (amino acids 115 to 126 in putative loop 3) of the 16 kDa-protein ductin, which forms subunit c of V-ATPases and is also supposed to be a part of gap junctions (AB5496, Chemicon International, USA), (2) a guinea-pig antiserum (Anti-V-ATPase a [19]) raised against an N-terminal region of subunit a of the *Manduca sexta* V-ATPase, kindly provided by B. Walz and O. Baumann (Potsdam, Germany), (3) a guinea-pig antiserum (Anti-Inx2 [18]) raised against the cytoplasmic loop of innexin 2, and (4) a guinea-pig antiserum (Anti-Inx3 [18]) raised against the C-terminus of innexin 3 from *Drosophila*, kindly provided by R. Bauer and M. Hoch (Bonn, Germany). A rabbit non-immune serum (NIS [11, 15–18]) was used as control.

Immunoblotting

Immunoblots were performed as described previously [11]. In short, homogenates of ovaries were sonicated and briefly boiled. The proteins were separated using 12 % SDS-PAGE and transferred to nitrocellulose membranes. Nonspecific binding sites were blocked with 5 % skimmed milk powder/PBS and the blots were incubated in Anti-ductin diluted 1:100 up to 1:500 with 1 % BSA/PBS. NIS diluted 1:200 was used as a control [11]. Subsequently, biotinylated goat-anti-rabbit IgG (Jackson, PA, USA; diluted 1:1000), streptavidin-peroxidase (Dianova, Germany; diluted 1:1000) and $\text{H}_2\text{O}_2/4$ -chloro-1-naphthol (Sigma, Germany) were applied, and photographs were

taken using a digital camera. Experiments were performed three times.

Indirect immunofluorescence preparations

Follicles were fixed for 30 min at 4 °C in 4 % formaldehyde dissolved in PBS, washed in PBS and blocked for 1 h at 20 °C with 2 % BSA/0,1 % Triton X-100 in PBS. Thereafter, the follicles were incubated overnight at 4 °C in PBS containing 0,5 % BSA/0,1 % Triton X-100 and two of the following antisera for double-labeling: Anti-ductin diluted 1:100, Anti-V-ATPase a diluted 1:1000, Anti-Inx2 diluted 1:20, Anti-Inx3 diluted 1:20. Controls were performed without antiserum and using NIS 1:200 [11, 18], respectively.

After washing six times for 10 min, the follicles were treated with goat-anti-rabbit-Cy3 (Jackson, USA; diluted 1:2000) and donkey-anti-guinea-pig-FP488 (FluoProbes/Interchim, France; diluted 1:100) for 1 h in PBS containing 0,5 % BSA/0,1 % Triton X-100. Washing was repeated six times and the nuclei were stained with 0,2 µg/ml DAPI (Sigma, Germany) in PBS for 3 min. Thereafter, the follicles were mounted in Fluoromount G (Interchim) and viewed, by using x20/0.5, x40/0.75 or x63/1.25oil objectives and the appropriate filter sets, either on a Zeiss Axiovert 200 wide-field fluorescence microscope (WFM), equipped with a Hamamatsu Orca ER camera, or they were mounted in glycerine/PBS 1:1 and viewed on a Leica DMRE laser-scanning microscope (LSM). Each experiment was repeated at least three times.

Results

Immunoblot analysis of Anti-ductin

Using immunoblots of *Drosophila* ovary preparations, we analysed the binding properties of the commercially available antiserum AB5496 (Anti-ductin). As described earlier for two other anti-ductin sera (AN2, prepared against *Nephrops* ductin and affinity-purified against *Drosophila* ductin, and AD16, prepared and affinity-purified against a peptide specific for the N-terminus of *Drosophila* ductin (cf. [11, 15–17])), Anti-ductin recognized a strong band at 29 kDa and weak bands at 16 and 45 kDa (Fig. 1a). No specific binding was obtained with NIS (cf. [11]). The 16 kDa-band represents the monomer of ductin and the other bands represent the putative dimer and trimer, respectively. Thus, the binding of Anti-ductin in indirect immunofluorescence preparations is assumed to be specific for the channel-forming protein ductin in *Drosophila* ovarian follicles.

Localization of ductin in indirect immunofluorescence preparations

Using Anti-ductin and laser-scanning microscopy (LSM), we investigated the distribution of ductin during the course of oogenesis (Fig. 1b–g). As shown previously using

the two other anti-ductin sera on sections prepared for immuno-fluorescence and immuno-electron microscopy [11, 16], the continuous as well as punctate distribution of ductin was found to change stage-specifically. In the following, we concentrate on ductin in specialized types of follicle cells (polar cells, border cells, stalk cells and centripetally migrating cells) and on some main features of its distribution relevant to the present comparative analysis.

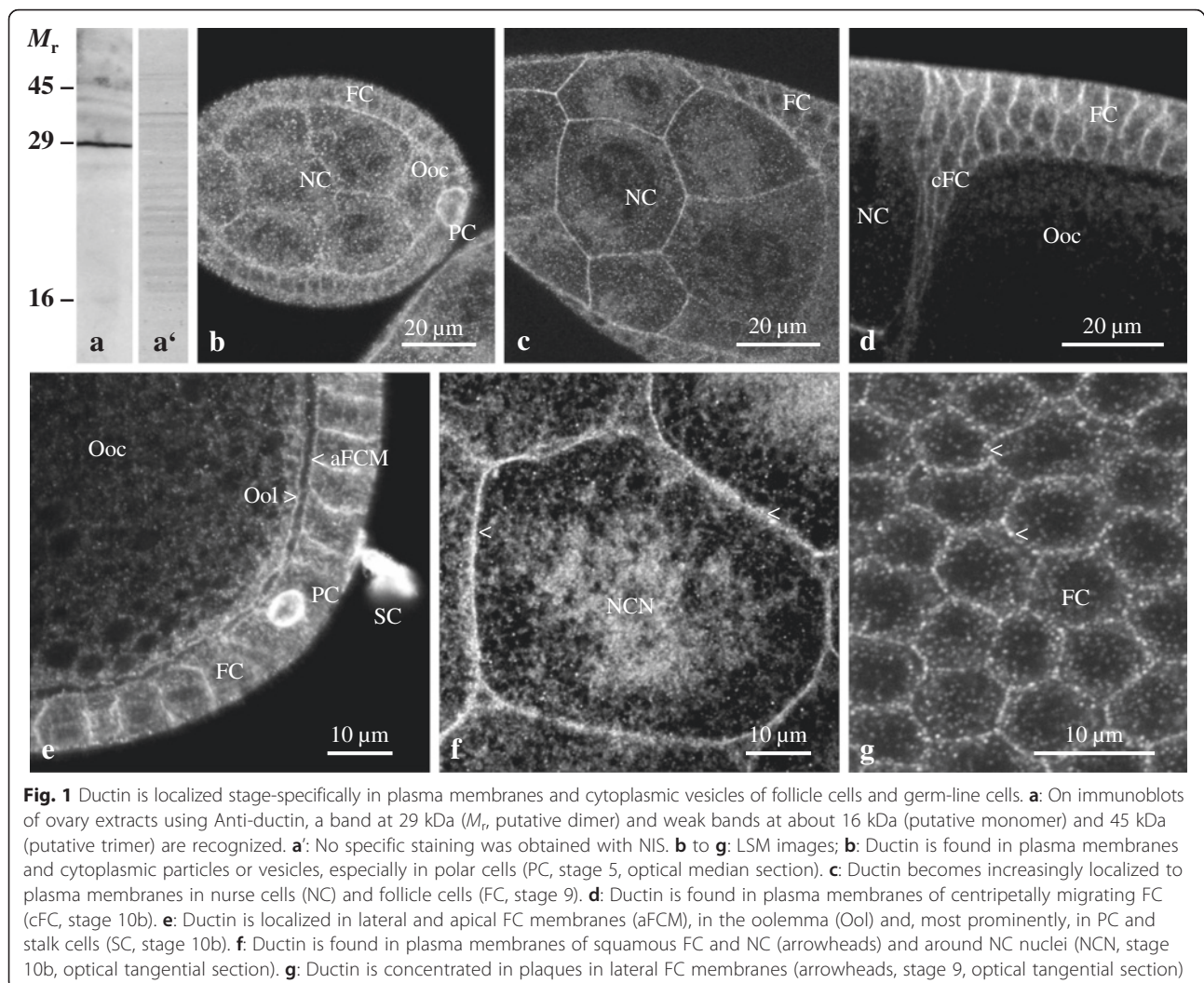
During previtellogenesis (up to stage 7), ductin is located in cytoplasmic particles or vesicles as well as in plasma membranes and is enriched in polar cells (Fig. 1b). Beginning with vitellogenesis, ductin increasingly becomes located in plasma membranes of nurse cells and follicle cells (Fig. 1c). During vitellogenesis (from stage 8 onward), ductin is located (1) in cytoplasmic vesicles, (2) in plasma membranes of centripetally migrating follicle cells (Fig. 1d), (3) in lateral and apical plasma membranes of columnar follicle cells, (4) in plasma membranes of squamous follicle cells and nurse cells as well as around nurse-cell nuclei, (5) in the oolemma, and (6), most prominently, in polar cells and stalk cells (Fig. 1e and f). Predominantly in lateral and apical follicle-cell membranes, ductin is localized in a punctate pattern resembling gap-junction plaques (Fig. 1g; (cf. [11])). No specific staining was found in control preparations without primary antiserum or using NIS (cf. [11, 18]).

Colocalization of ductin with V-ATPase subunit a

Since ductin forms subunit c of V-ATPases, it is expected to localize, at least in part, together with subunit a in the same cellular regions. We analysed follicles double-labeled with Anti-ductin and Anti-V-ATPase a using wide-field fluorescence microscopy (WFM, Fig. 2). Stage-specifically, ductin and subunit a were found colocalized in plasma membranes and in the cytoplasm of follicle cells and germ-line cells, especially in cytoplasmic particles or vesicles.

During early previtellogenesis, ductin and subunit a co-occur (1) in polar cells, (2) in lateral and apical follicle-cell membranes and (3) in the cytoplasm of follicle cells, nurse cells and the oocyte. However, while ductin was found to be more prominent in stalk cells, the distinct particles or vesicles in germ-line cells were found to contain predominantly subunit a (Fig. 2a). During late previtellogenesis, both ductin and subunit a are enriched in apical follicle-cell membranes. On the other hand, ductin was found to be more prominent in posterior polar cells and in stalk cells, while subunit a was more prominent in the cytoplasm of follicle cells, nurse cells and the oocyte (Fig. 2b).

During vitellogenesis, ductin and subunit a co-occur especially in columnar and squamous follicle cells and in



border cells. In columnar follicle cells, the amount is usually higher on one side of the follicle (Fig. 2c), namely the ventral side (cf. [19]). Here, ductin and subunit a are colocalized in the plasma membranes and in the cytoplasm while, in squamous follicle cells, they are colocalized in vesicles (Fig. 2d). On the other hand, ductin is enriched in nurse-cell membranes, while subunit a is enriched in border cells (Fig. 2c) as well as in nuclear particles or vesicles in follicle cells (Fig. 2e). During late vitellogenesis, ductin and subunit a are colocalized in cytoplasmic vesicles in most of the follicle cells, whereas, in the cytoplasm and nuclei of anterodorsal follicle cells, predominantly subunit a was found (Fig. 2f). Taken together, ductin and subunit a are stage-specifically either colocalized or separately enriched in different cellular regions of germ-line and soma cells. Frequently, ductin is more prominent in plasma membranes, while subunit a is more prominent in cytoplasmic and nuclear particles or vesicles (for summary, see Fig. 5a and a').

Colocalization of ductin with innexins

Since ductin is known to form intercellular communication channels [11, 15, 31], it might localize, in part, together with members of the channel-forming innexin family in the same cellular regions [27–30]. In *Drosophila* ovarian follicles, the localization of innexins 1 to 4 has been described in detail previously using LSM and WFM [18]. For the present comparative analysis, we concentrate on some main features concerning Inx2 and Inx3, that both showed distribution patterns similar to ductin.

Ductin and Inx2

We analysed follicles double-labeled with Anti-ductin and Anti-Inx2 using WFM (Fig. 3). During previtellogenesis (Fig. 3a, b), ductin and Inx2 co-occur (1) in lateral and apical follicle-cell membranes and (2) in the cytoplasm of follicle cells, nurse cells and the oocyte. However, ductin was found to be enriched (1) in nurse-cell membranes and (2) in the oolemma as well as (3) in

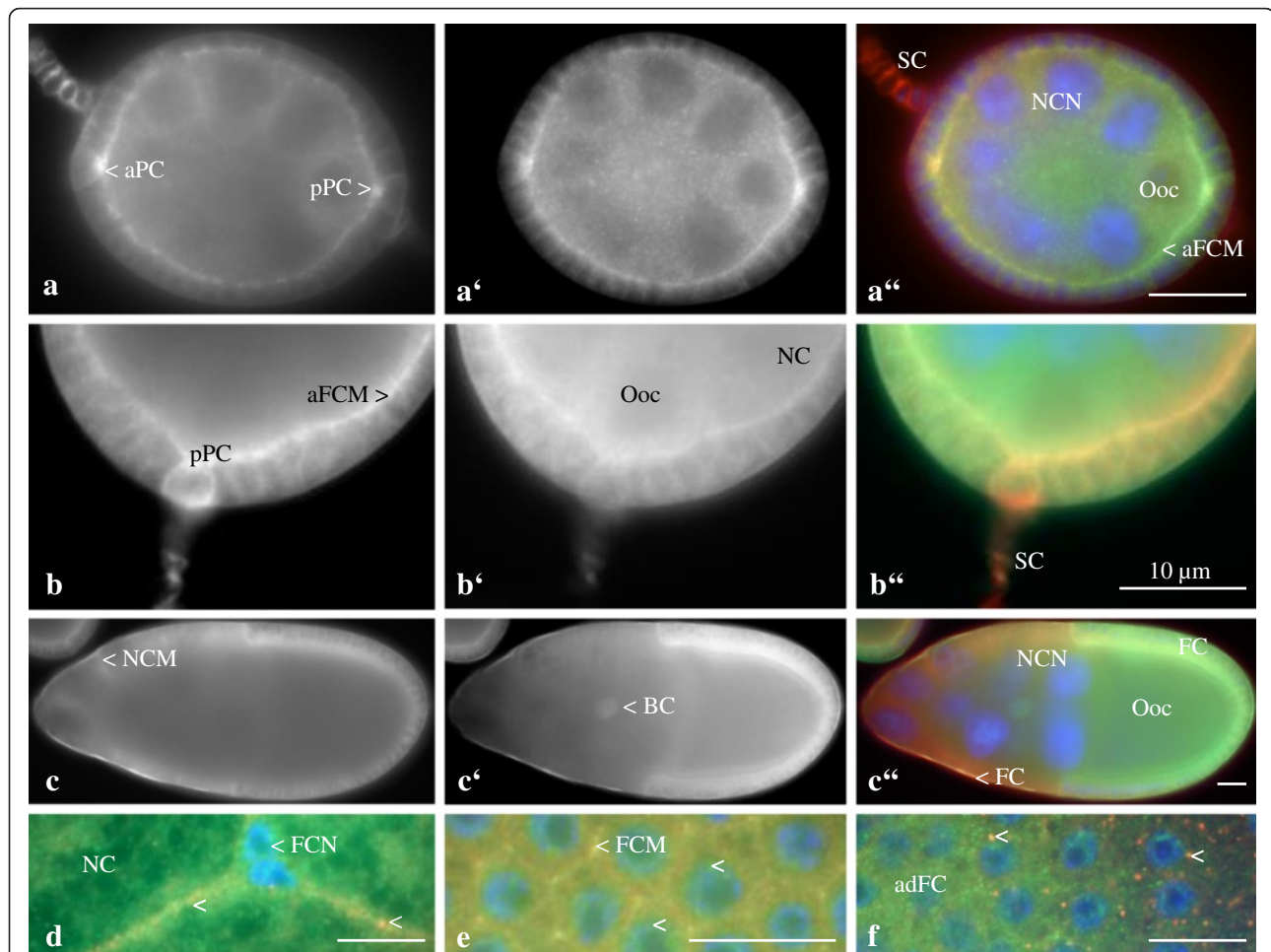


Fig. 2 Ductin and V-ATPase subunit a are partly colocalized in plasma membranes and in the cytoplasm of follicle cells and germ-line cells, often in cytoplasmic particles or vesicles. **a** to **c**: Ductin (subunit c); **a'** to **c'**: V-ATPase subunit a; **a''** to **c''** and **d** to **f**: merged images, ductin (red), V-ATPase subunit a (green) and colocalization of both (yellow), WFM. Nuclei were stained with DAPI. No specific staining was obtained with NIS (cf. inset in Fig. 4b). In young previtellogenic follicles (**a** to **a''**, stage 4), ductin and V-ATPase subunit a co-occur in anterior and posterior polar cells (aPC, pPC), in lateral and apical follicle-cell membranes (aFCM), and in the cytoplasm of FC, nurse cells (NCN, NC nucleus) and the oocyte (Ooc). While cytoplasmic particles or vesicles containing predominantly V-ATPase subunit a can be observed (**a'**), ductin is enriched in stalk cells (SC). In older previtellogenic follicles (**b** to **b''**, stage 6), ductin and V-ATPase subunit a are enriched in aFCM. While ductin is found in higher amounts in pPC and SC, V-ATPase subunit a is found in higher amounts in the cytoplasm of FC, NC and Ooc (**b'**). In vitellogenic follicles (**c** to **c''**, stage 10a), ductin and V-ATPase subunit a are colocalized especially in columnar and squamous FC. While ductin is enriched in NC membranes (NCM), V-ATPase subunit a is enriched in border cells (BC). **d**: Ductin and V-ATPase subunit a are colocalized in particles or vesicles (arrowheads, yellow) in squamous FC (FCN, FC nucleus, stage 10b, surface view). **e**: Ductin and V-ATPase subunit a are colocalized in lateral FC membranes (FCM) as well as in the cytoplasm (yellow, stage 10b, surface view). Particles or vesicles containing V-ATPase subunit a are found in FCN (arrowheads, green). **f**: In older follicles (stage 12, surface view), ductin and V-ATPase subunit a are colocalized in particles or vesicles in most of the FC (arrowheads, yellow) while, in anterodorsal FC (adFC), predominantly V-ATPase subunit a is found in the cytoplasm and in the nuclei (green vesicles). Scale bars, 10 μ m

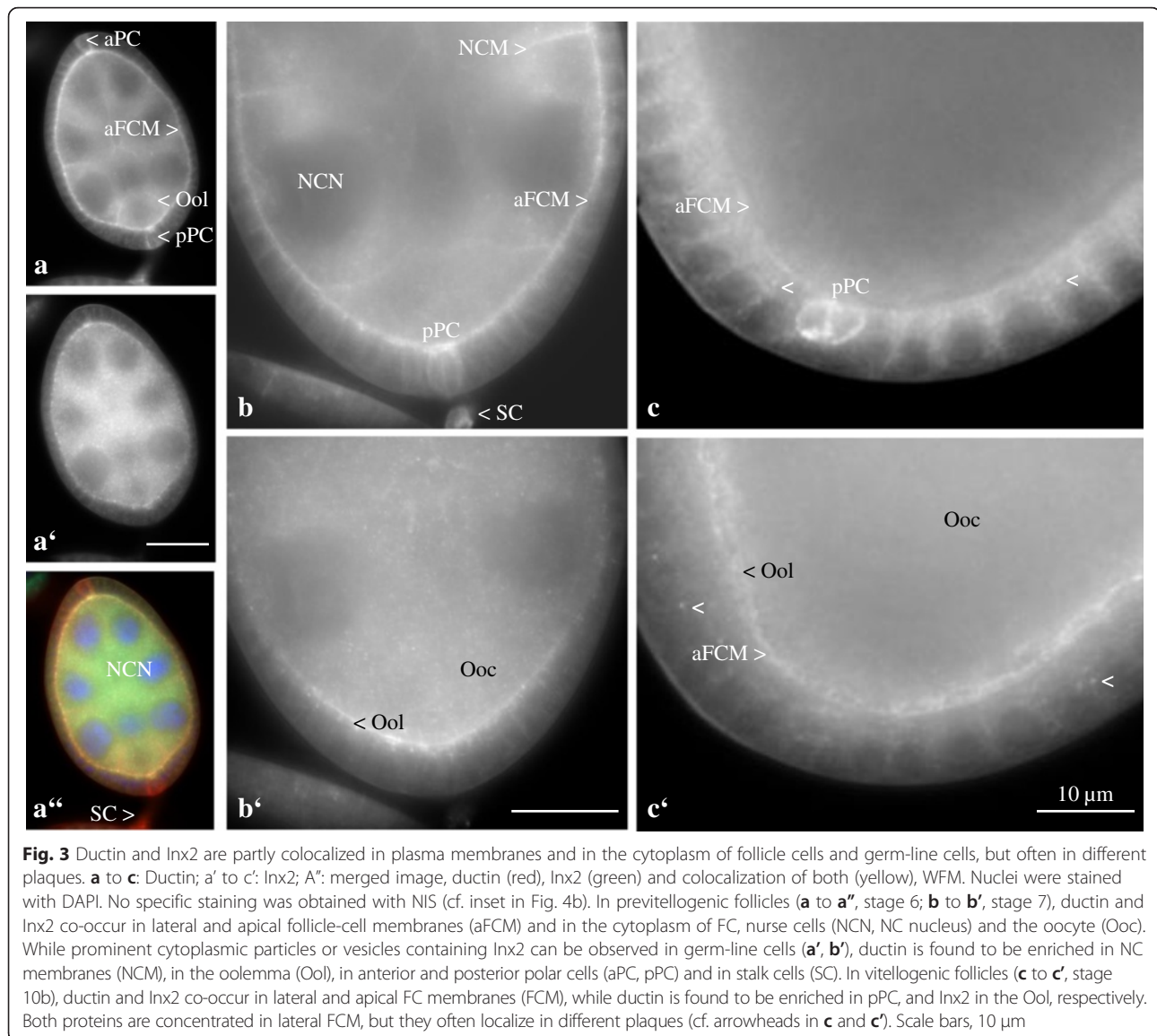
polar cells and (4) in stalk cells, whereas cytoplasmic particles or vesicles containing predominantly *Inx2* were observed in germ-line cells.

Also during vitellogenesis (Fig. 3c), ductin and *Inx2* co-occur in lateral and apical follicle-cell membranes. On the other hand, ductin is still enriched in posterior polar cells, while *Inx2* is now enriched in the oolemma. In lateral follicle-cell membranes, both proteins are often found concentrated in different plaques that are presumed

to represent different gap junctions (arrowheads in Fig. 3c and c').

Ductin and *Inx3*

Follicles double-labeled with Anti-ductin and Anti-*Inx3* were also analysed using WFM (Fig. 4). In previtellogenic and early vitellogenic stages (Fig. 4a), ductin and *Inx3* co-occur in follicle-cell membranes. However, ductin is more prominent (1) in nurse-cell membranes and (2) in



the oolemma as well as (3) in polar cells, (4) in stalk cells and (5) in the cytoplasm of germ-line cells. On the other hand, Inx3 was found to be enriched around nurse-cell nuclei and in lateral plasma membranes of the prospective centripetally migrating follicle cells.

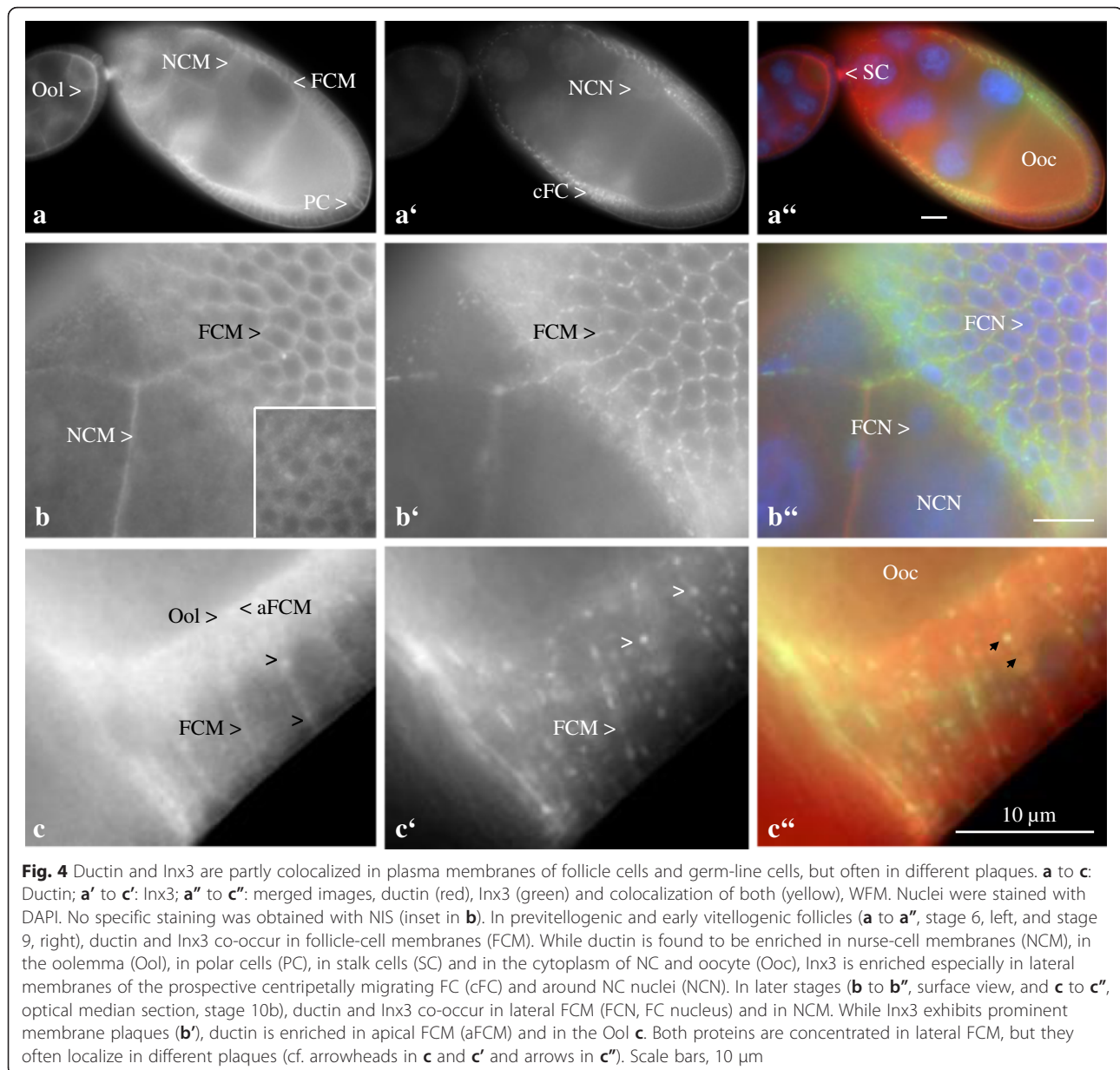
In mid to late vitellogenic stages (Fig. 4b, c), ductin and Inx3 co-occur in lateral follicle-cell membranes and in nurse-cell membranes. While ductin is enriched in apical follicle-cell membranes and in the oolemma, Inx3 forms prominent plaques in lateral follicle-cell membranes. Here, ductin and Inx3 are often found concentrated in different plaques that are presumed to represent different gap junctions (arrowheads in Fig. 4c and c' and arrows in c'').

Taken together, ductin and both innexins are stage-specifically either colocalized or separately enriched in

different cellular regions of germ-line and soma cells. Frequently, ductin shows a rather continuous membrane distribution, while the distribution of both innexins is more punctate (for summary, see Fig. 5b, b' and c, c').

Discussion

The channel-forming protein ductin has been found in both V-ATPases and gap-junctional structures [31]. In V-ATPases, it forms the proteolipid component or subunit c, whereof the C- and N-termini as well as loop 2 are located either on the vacuolar or on the extracellular side of the membrane. In gap-junctional structures, where ductin is supposed to form membrane channels, these regions are located on the cytoplasmic side of the membrane [33, 34]. Using light and electron microscopy, it has been shown that antibodies against ductin bind to

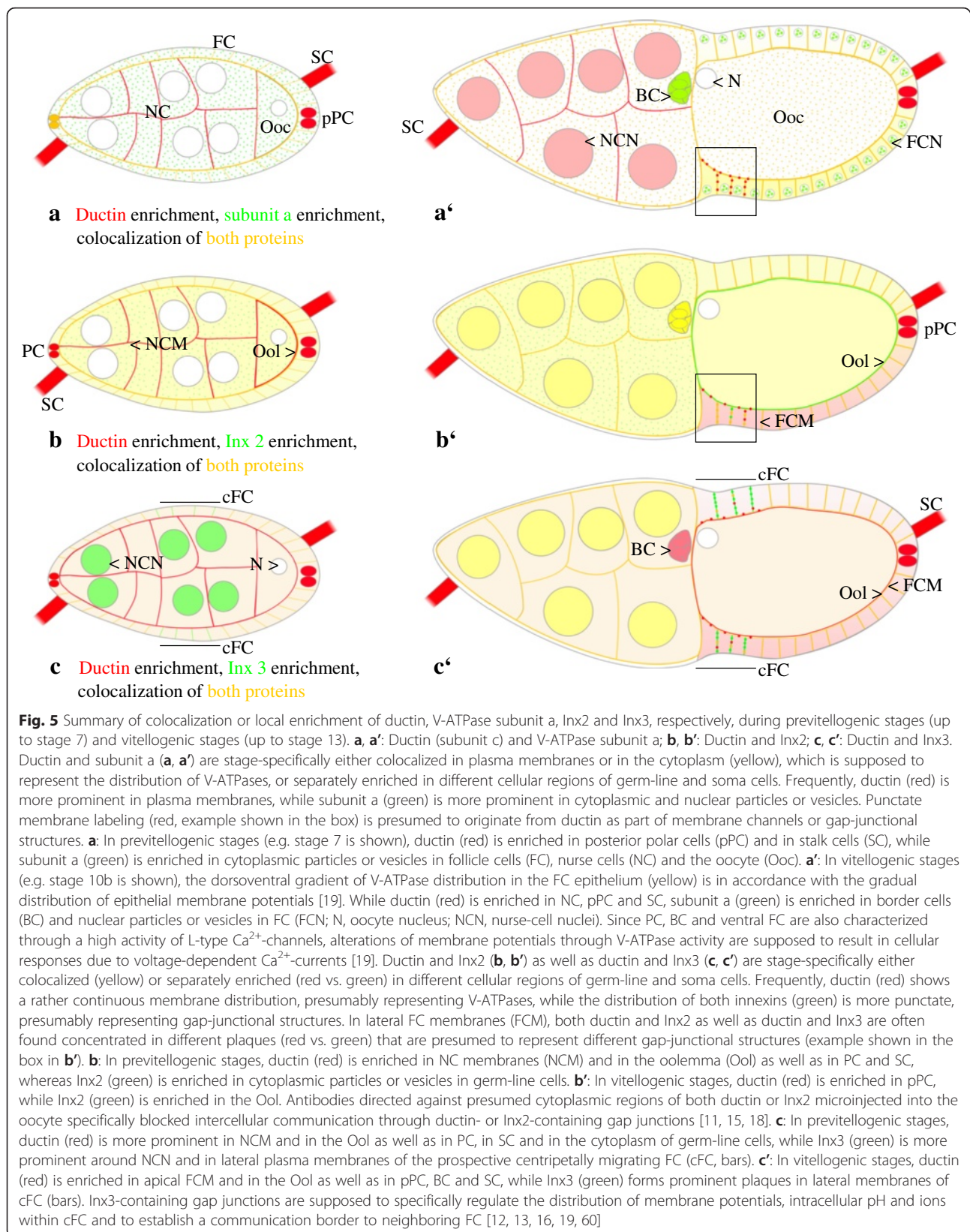


antigens located in the cytoplasm and in plasma membranes of various *Drosophila* tissues, especially of ovarian follicles and embryos [11, 15–17]. In part, cytoplasmic labeling might represent storage or transport of ductin. However, since V-ATPases are required for the acidification of organelles (e.g. [35]), cytoplasmic labeling is likely to also represent secretory vesicles, lysosomes or endosomes in which ligand-receptor complexes become dissociated (e.g. [16, 36]).

On the other hand, V-ATPases coupled to secondary active antiport mechanisms are known to energize transport processes across plasma membranes (e.g. [37]). These pumps are expected to exert direct or indirect influence on intracellular pH and membrane potentials. In the

follicle-cell epithelium of *Drosophila*, antibodies against both subunits a and c revealed an asymmetric distribution of V-ATPases which is in accordance with the gradual dorsoventral distribution of epithelial membrane potentials [19]. This observation points to a possible involvement of V-ATPases in the regulation or maintenance of the spatial coordinates during oogenesis, a phenomenon that has been observed in other systems (e.g. [38–40]). Moreover, V-ATPases are also known to be involved in the regulation of cell division, migration and differentiation (e.g. [41, 42]).

Labeling of plasma membranes with antibodies against ductin was either continuous or punctate (cf. [11, 16, 19]). Continuous labeling is presumed to originate from ductin



as part of V-ATPases, while punctate labeling might originate from ductin as part of gap-junctional structures. By microinjection experiments, it has been shown that antibodies directed against presumed cytoplasmic regions of ductin specifically block intercellular communication and exert adverse influence on oogenesis as well as on embryogenesis [11, 15].

Interestingly, ductin labeling was prominent in both stalk cells and polar cells that derive from a common cell population in the germarium. Polar cells are known to control stalk formation as well as axis formation, they recruit the border cells and they express various cell-specific markers [43, 44].

Evidence amounts that ductin, or subunit c, is a multi-functional channel protein. It has been observed to form Ca^{2+} -inducible membrane pores that are permeable to hydrophilic molecules [45]. It has also been demonstrated that hexamers of subunit c form stacked rings containing an intercellular channel [46, 47]. And, in addition, ductin seems to play a role in the process of vesicle-membrane fusion [48]. The present study lends further support to the notion that ductin might be engaged in several of these functions also during *Drosophila* oogenesis.

Although various proteins have been described to be part of *Drosophila* gap junctions (cf. [15, 27]), members of the innexin family are assumed to be the main invertebrate gap-junction proteins [29, 30, 49]. In the *Drosophila* ovary, the mRNAs of innexins 1, 2, 3, 4 and (to a minor extent) 7 have been detected [50]. Using various antibodies against innexins (Inx), Inx1 was found to be predominantly located in the baso-lateral domain of follicle cells, whereas Inx2 is positioned apico-laterally as well as apically between follicle cells and germ-line cells. Inx3 was observed laterally in follicle cells and also in nurse cells, and Inx4 was detected in the oolemma up to stage 8 and in nurse-cell membranes up to stage 12. While Inx2 and Inx3 are colocalized between somatic cells, Inx2 and Inx4 are colocalized between somatic cells and germ-line cells [18].

Microinjected antibodies directed against presumed cytoplasmic regions of Inx2 specifically blocked intercellular communication and lead to inhibitory effects on oogenesis that were similar to those obtained with antibodies directed against presumed cytoplasmic regions of ductin [18]. Even during spermatogenesis, Inx2 was found to be involved in soma-to-germ line intercellular communication [51]. Moreover, it has been demonstrated that, within the germarium, Inx2 supports germ-cell survival, cyst formation and early follicle development [52].

During embryogenesis, Inx2 was shown to participate in organizing epithelia by interacting with core proteins of adherens and septate junctions, and it has been presumed that Inx2 exerts its structural functions indirectly

through the formation of channels for intercellular communication [53–56]. Recently, Inx2 has also been identified as a regulator of eye development [57].

Contrary to Inx2, Inx3 shows a striking non-uniform distribution in the follicle-cell epithelium [19]: it is enriched in the lateral membranes of centripetally migrating follicle cells. This indicates distinct coupling conditions between these cells concerning e.g. regulatory signals, membrane potentials or intracellular pH. Moreover, Inx3 has been described to be involved in maintaining tissue integrity in response to tension [58], a feature that is particularly important for migrating cells. The spatial restriction of specialized intercellular channels and the independent regulation of their permeability is suspected to provide mechanisms to generate or maintain boundaries between distinct follicle-cell populations [19]. By the use of various proteins in either homomeric, heteromeric or heterotypic combinations, a wealth of different gap-junction channels might be formed (cf. [18]).

Compared to neighboring cells, the centripetally migrating follicle cells are specialized in several respects: (1) they are more acidic and more depolarized [19], (2) they possess higher amounts of active voltage-dependent L-type Ca^{2+} -channels [19] (becoming activated under depolarized conditions [59]), (3) they contain higher Ca^{2+} -concentrations [60], (4) they possess higher amounts of Na^+ , K^+ -ATPase [13, 16] (becoming stimulated under alkaline conditions [10]), and (5) they remain coupled via gap junctions to the oocyte when neighboring cells are already uncoupled [12].

The permeability of innexin channels in general has been reported to be sensitive to membrane potential, intracellular pH, K^+ - and Ca^{2+} -concentrations (e.g. [61, 62]). Gap-junctional communication between oocyte and follicle cells was found to be inhibited by low extracellular pH, by low extracellular K^+ -concentrations (leading to hyperpolarization [21, 22]), and by high Ca^{2+} -concentrations in the oocyte [12]. Taken together, these findings support the notion that intercellular communication in *Drosophila* ovarian follicles is precisely regulated.

Similar to ductin and to the pannexins in vertebrates, innexins have also been found to form non-junctional channels (innexons) allowing the secretion of small molecules, including ATP, into the extracellular space [47, 62–64]. Innexons were observed to open in response to mechanical stress, depolarization, high intracellular Ca^{2+} - and high extracellular K^+ -concentrations, and to close due to low intracellular pH [62, 63].

Conclusions

In recent years, the origin as well as the physiological and cellular functions of bioelectric phenomena have attracted growing attention and, thus, their meaning becomes more and more elucidated (e.g. [4, 65–68]).

Accordingly, the distribution and activity patterns of membrane channels, like ductin and innexins, during the course of *Drosophila* oogenesis are supposed to contribute to developmentally important bioelectric signals [19].

Since ductin shows overlapping distribution patterns with both *Inx2* and *Inx3* (for summary, see Fig. 5), these proteins might be, in part, located within the same gap-junctional structures, especially between neighboring follicle cells. However, the analysed proteins are often found concentrated in different membrane plaques which supports the notion of different gap junctions with a large variety of protein compositions.

Particularly, ductin is enriched in polar cells and stalk cells, while *Inx2* is enriched in the oolemma, and *Inx3* is enriched in centripetally migrating follicle cells, but the other proteins are not absent from these regions. Since ductin is part of a proton pump and, like the innexins, can form junctional as well as non-junctional membrane channels, a plethora of cellular functions could be realized by using only the three proteins analysed in the present study.

Abbreviations

a, anterior; ad, anterodorsal; BC, border cells; BSA, bovine serum albumine; c, centripetally migrating; DAPI, 4',6-diamidino-2-phenylindole; DMSO, dimethyl sulfoxide; FC, follicle cells; *Inx2*, innexin 2; *Inx3*, innexin 3; LSM, laser-scanning microscopy; M, membrane; N, nucleus; NC, nurse cells; NIS, non-immune serum; Ooc, oocyte; Ool, oolemma; p, posterior; PBS, phosphate buffered saline; PC, polar cells; SC, stalk cells; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel-electrophoresis; WFM, wide-field microscopy

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Availability of data and materials

The dataset supporting the conclusions of this article is included within the article.

Authors' contributions

JL carried out the experiments under the supervision of JB. JB conceived the study, reviewed the data and wrote the manuscript. Both authors read and approved the final version.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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References

- McCaig CD, Rajnicek AM, Song B, Zhao M. Controlling cell behaviour electrically: current views and future potential. *Physiol Rev.* 2005;85:943–78.

- Campetelli A, Bonazzi D, Minc N. Electrochemical regulation of cell polarity and the cytoskeleton. *Cytoskeleton.* 2012;69:601–12.
- Adams DS, Levin M. Endogenous voltage gradients as mediators of cell-cell communication: strategies for investigating bioelectrical signals during pattern formation. *Cell Tissue Res.* 2013;352:95–122.
- Chang F, Minc N. Electrochemical control of cell and tissue polarity. *Annu Rev Cell Dev Biol.* 2014;30:317–36.
- Levin M. Endogenous bioelectric networks store non-genetic patterning information during development and regeneration. *J Physiol.* 2014;592:2295–305.
- Bohrmann J, Dorn A, Sander K, Gutzeit H. The extracellular electrical current pattern and its variability in vitellogenic *Drosophila* follicles. *J Cell Sci.* 1986a;81:189–206.
- Bohrmann J, Huebner E, Sander K, Gutzeit H. Intracellular electrical potential measurements in *Drosophila* follicles. *J Cell Sci.* 1986b;81:207–21.
- Bohrmann J, Gutzeit HO. Evidence against electrophoresis as the principal mode of protein transport in vitellogenic ovarian follicles of *Drosophila*. *Development.* 1987;101:279–88.
- Bohrmann J. In vitro culture of *Drosophila* ovarian follicles: the influence of different media on development, RNA synthesis, protein synthesis and potassium uptake. *Roux's Arch Dev Biol.* 1991a;199:315–26.
- Bohrmann J. Potassium uptake into *Drosophila* ovarian follicles: relevance to physiological and developmental processes. *J Insect Physiol.* 1991b;37:937–46.
- Bohrmann J. Antisera against a channel-forming 16 kDa protein inhibit dye-coupling and bind to cell membranes in *Drosophila* ovarian follicles. *J Cell Sci.* 1993;105:513–8.
- Bohrmann J, Haas-Assenbaum A. Gap junctions in ovarian follicles of *Drosophila melanogaster*: inhibition and promotion of dye-coupling between oocyte and follicle cells. *Cell Tissue Res.* 1993;273:163–73.
- Bohrmann J, Heinrich U-R. Localisation of potassium pumps in *Drosophila* ovarian follicles. *Zygote.* 1994;2:189–99.
- Bohrmann J, Schill S. Cytoplasmic transport in *Drosophila* ovarian follicles: the migration of microinjected fluorescent probes through intercellular bridges depends neither on electrical charge nor on external osmolarity. *Int J Dev Biol.* 1997;41:499–507.
- Bohrmann J, Lämmel H. Microinjected antisera against ductin affect gastrulation in *Drosophila melanogaster*. *Int J Dev Biol.* 1998;42:709–21.
- Bohrmann J, Braun B. Na, K-ATPase and V-ATPase in ovarian follicles of *Drosophila melanogaster*. *Biol Cell.* 1999;91:85–98.
- Bohrmann J, Bonafede A. Tissue-specific distribution and variation of the channel-forming protein ductin during development of *Drosophila melanogaster*. *Int J Dev Biol.* 2000;44:883–90.
- Spradling J, Zimmermann J. Gap junctions in the ovary of *Drosophila melanogaster*: localization of innexins 1, 2, 3 and 4 and evidence for intercellular communication via innexin-2 containing channels. *BMC Dev Biol.* 2008;8:111.
- Krüger J, Bohrmann J. Bioelectric patterning during oogenesis: stage-specific distribution of membrane potentials, intracellular pH and ion-transport mechanisms in *Drosophila* ovarian follicles. *BMC Dev Biol.* 2015;15:1.
- Overall R, Jaffe LF. Patterns of ionic currents through *Drosophila* follicles and eggs. *Dev Biol.* 1985;108:102–19.
- Sun YA, Wyman RJ. Reevaluation of electrophoresis in the *Drosophila* egg chamber. *Dev Biol.* 1993;155:206–15.
- Munley SM, Kinzler S, Lizzano R, Woodruff RI. Fractional contribution of major ions to the membrane potential of *Drosophila melanogaster* oocytes. *Arch Insect Biochem Physiol.* 2009;70:230–43.
- Spradling A. Developmental genetics of oogenesis. In: Bate M, Martinez Arias A, editors. *The Development of Drosophila melanogaster*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 1993. p. 1–70.
- Riechmann V, Ephrussi A. Axis formation during *Drosophila* oogenesis. *Curr Opin Genet Dev.* 2001;11:374–83.
- Mahowald AP. Ultrastructural observations on oogenesis in *Drosophila*. *J Morphol.* 1972;137:29–48.
- Giorgi F, Postlethwait JH. Development of gap junctions in normal and mutant ovaries of *Drosophila melanogaster*. *J Morphol.* 1985;185:115–29.
- Phelan P. Gap junction communication in invertebrates: The innexin gene family. *Curr Top Membranes.* 2000;49:389–422.
- Phelan P, Starich TA. Innexins get into the gap. *Bioessays.* 2001;23:388–96.
- Phelan P. Innexins: members of an evolutionarily conserved family of gap-junction proteins. *Biochim Biophys Acta - Biomembranes.* 1711;2005:225–45.
- Bauer R, Löer B, Ostrowski K, Martini J, Weimbs A, Lechner H, Hoch M. Intercellular communication: the *Drosophila* innexin multiprotein family of gap junction proteins. *Chem Biol.* 2005;12:515–26.

31. Finbow ME, Pitts JD. Structure of the ductin channel. *Biosci Rep*. 1998;18:287–97.
32. Robb JA. Maintenance of imaginal discs of *Drosophila melanogaster* in chemically defined media. *J Cell Biol*. 1969;41:876–85.
33. Finbow ME, Goodwin SF, Meagher L, Lane NJ, Keen J, Findlay JBC, Kaiser K. Evidence that the 16 kDa proteolipid (subunit c) of the vacuolar H⁺-ATPase and ductin from gap junctions are the same polypeptide in *Drosophila* and *Manduca*: molecular cloning of the Vha 16 k gene from *Drosophila*. *J Cell Sci*. 1994;107:1817–24.
34. Dunlop J, Jones PC, Finbow ME. Membrane insertion and assembly of ductin: a polytopic channel with dual orientations. *EMBO J*. 1995;14:3609–16.
35. Kawasaki-Nishi S, Nishi T, Forgac M. Proton translocation driven by ATP hydrolysis in V-ATPases. *FEBS Lett*. 2003;545:76–85.
36. Vaccari T, Duchi S, Cortese K, Tacchetti C, Bilder D. The vacuolar ATPase is required for physiological as well as pathological activation of the Notch receptor. *Development*. 2010;137:1825–32.
37. Harvey WR, Wieczorek H. Animal plasma membrane energization by chemiosmotic H⁺ V-ATPases. *J Exp Biol*. 1997;200:203–16.
38. Adams DS, Robinson KR, Fukumoto T, Yuan S, Albertson RC, Yelick P, Kuo L, McSweeney M, Levin M. Early, H⁺-V-ATPase-dependent proton flux is necessary for consistent left-right patterning of non-mammalian vertebrates. *Development*. 2006;133:1657–71.
39. Vandenberg LN, Morrie RD, Adams DS. V-ATPase-dependent ectodermal voltage and pH regionalization are required for craniofacial morphogenesis. *Dev Dyn*. 2011;240:1889–904.
40. Vandenberg LN, Adams DS, Levin M. Normalized shape and location of perturbed craniofacial structures in the *Xenopus* tadpole reveal an innate ability to achieve correct morphology. *Dev Dyn*. 2012;241:863–78.
41. Nishi T, Forgac M. The vacuolar (H⁺)-ATPases – nature's most versatile proton pumps. *Nat Rev Mol Cell Biol*. 2002;3:94–103.
42. Nuccitelli R. Endogenous electric fields in embryos during development, regeneration and wound healing. *Radiat Prot Dosimetry*. 2003;106:375–83.
43. Ruohola H, Bremer KA, Baker D, Swedlow JR, Jan LY, Jan YN. Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in *Drosophila*. *Cell*. 1991;66:433–49.
44. Grammont M, Irvine KD. Organizer activity of the polar cells during *Drosophila* oogenesis. *Development*. 2002;129:5131–40.
45. Morel N. Neurotransmitter release: the dark side of the vacuolar-H⁺ATPase. *Biol Cell*. 2003;95:453–7.
46. Clare DK, Orlova EV, Finbow MA, Harrison MA, Findlay JBC, Saibil HR. An expanded and flexible form of the vacuolar ATPase membrane sector. *Structure*. 2006;14:1149–56.
47. Rawson S, Phillips C, Huss M, Tiburcy F, Wieczorek H, Trinick J, Harrison MA, Muench SP. Structure of the vacuolar H⁺-ATPase rotary motor reveals new mechanistic insights. *Structure*. 2015;23:461–71.
48. Vavassori S, Mayer A. A new life for an old pump: V-ATPase and neurotransmitter release. *J Cell Biol*. 2014;205:7–9.
49. Hasegawa DK, Turnbull MW. Recent findings in evolution and function of insect innexins. *FEBS Lett*. 2014;588:1403–10.
50. Stebbings LA, Todman MG, Phillips R, Greer CE, Tam J, Phelan P, Jacobs K, Bacon JP, Davies JA. Gap junctions in *Drosophila*: developmental expression of the entire innexin gene family. *Mech Dev*. 2002;113:197–205.
51. Smendziuk CM, Messenberg A, Vogl AW, Tanentzapf G. Bi-directional gap junction-mediated soma-germline communication is essential for spermatogenesis. *Development*. 2015;142:2598–609.
52. Mukai M, Kato H, Hira S, Nakamura K, Kita H, Kobayashi S. Innexin2 gap junctions in somatic support cells are required for cyst formation and for egg chamber formation in *Drosophila*. *Mech Dev*. 2011;128:510–23.
53. Bauer R, Lehmann C, Fuss B, Eckardt F, Hoch M. The *Drosophila* gap junction channel gene *innexin 2* controls foregut development in response to Wingless signalling. *J Cell Sci*. 2002;115:1859–67.
54. Bauer R, Lehmann C, Martini J, Eckardt F, Hoch M. Gap junction channel protein innexin 2 is essential for epithelial morphogenesis in the *Drosophila* embryo. *Mol Biol Cell*. 2004;15:2992–3004.
55. Bauer R, Weimbs A, Lechner H, Hoch M. DE-cadherin, a core component of the adherens junction complex modifies subcellular localization of the *Drosophila* gap junction protein innexin 2. *Cell Commun Adhes*. 2006;13:103–14.
56. Lehmann C, Lechner H, Löer B, Knieps M, Herrmann S, Famulok M, Bauer R, Hoch M. Heteromerization of innexin gap junction proteins regulates epithelial tissue organization in *Drosophila*. *Mol Biol Cell*. 2006;17:1676–85.
57. Richard M, Hoch M. *Drosophila* eye size is determined by innexin 2-dependent decapentaplegic signalling. *Dev Biol*. 2015;408:26–40.
58. Giuliani F, Giuliani G, Bauer R, Rabouille C. Innexin 3, a new gene required for dorsal closure in *Drosophila* embryo. *PLoS One*. 2013;8:e69212.
59. Hockerman GH, Peterson BZ, Johnson BD, Catterall WA. Molecular determinants of drug binding and action on L-type calcium channels. *Annu Rev Pharmacol Toxicol*. 1997;37:361–96.
60. Heinrich U-R, Gutzeit HO. Characterization of cation-rich follicle cells in vitellogenic follicles of *Drosophila melanogaster*. *Differentiation*. 1985;28:237–43.
61. Stebbings LA, Todman MG, Phelan P, Bacon JP, Davies JA. Two *Drosophila* innexins are expressed in overlapping domains and cooperate to form gap-junction channels. *Mol Biol Cell*. 2000;11:2459–70.
62. Dahl G, Muller KJ. Innexin and pannexin channels and their signaling. *FEBS Lett*. 2014;588:1396–402.
63. Bao L, Samuels S, Locovei S, Macagno ER, Muller KJ, Dahl G. Innexins form two types of channels. *FEBS Lett*. 2007;581:5703–8.
64. Luo K, Turnbull MW. Characterization of nonjunctional hemichannels in caterpillar cells. *J Insect Sci*. 2011;11:6.
65. Levin M. Gap junctional communication in morphogenesis. *Progr Biophys Mol Biol*. 2007;94:186–206.
66. Levin M. Molecular bioelectricity in developmental biology: new tools and recent discoveries. *Bioessays*. 2012;34:205–17.
67. Levin M, Stevenson CG. Regulation of cell behavior and tissue patterning by bioelectric signals: challenges and opportunities for biomedical engineering. *Annu Rev Biomed Eng*. 2012;14:295–323.
68. Levin M. Molecular bioelectricity: how endogenous voltage potentials control cell behavior and instruct pattern regulation in vivo. *Mol Biol Cell*. 2014;25:3835–50.

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