Bioelectrical and cytoskeletal patterns correlate with altered axial polarity in the follicular epithelium of the Drosophila mutant gurken

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Abstract

Background: Bioelectrical signals are known to be involved in the generation of cell and tissue polarity as well as in cytoskeletal dynamics. The epithelium of Drosophila ovarian follicles is a suitable model system for studying connections between electrochemical gradients, patterns of cytoskeletal elements and axial polarity. By interactions between soma and germline cells, the transforming growth factor-α homolog Gurken (Grk) establishes both the anteroposterior and the dorsoventral axis during oogenesis.

Results: In the follicular epithelium of the wild-type (wt) and the polarity mutant grk, we analysed stage-specific gradients of membrane potentials (V_memb) and intracellular pH (pH_i) using the potentiometric dye DiBAC_4(3) and the fluorescent pH-indicator 5-CFDA,AM, respectively. In addition, we compared the cytoskeletal organisation in the follicular epithelium of wt and grk using fluorescent phalloidin and an antibody against acetylated α-tubulin. Corresponding to impaired polarity in grk, the slope of the anteroposterior V_memb-gradient in stage S9 is significantly reduced compared to wt. Even more striking differences in V_memb- and pH_i-patterns become obvious during stage S10B, when the respective dorsoventral gradients are established in wt but not in grk. Concurrent with bioelectrical differences, wt and grk exhibit differences concerning cytoskeletal patterns in the follicular epithelium. During all vitellogenic stages, basal microfilaments in grk are characterised by transversal alignment, while wt-typical condensations in centripetal follicle cells (S9) and in dorsal centripetal follicle cells (S10B) are absent. Moreover, in grk, longitudinal alignment of microtubules occurs throughout vitellogenesis in all follicle cells, whereas in wt, microtubules in mainbody and posterior follicle cells exhibit a more cell-autonomous organisation. Therefore, in contrast to wt, the follicular epithelium in grk is characterised by missing or shallower electrochemical gradients and by more coordinated transcellular cytoskeletal patterns.

Conclusions: Our results show that bioelectrical polarity and cytoskeletal polarity are closely linked to axial polarity in both wt and grk. When primary polarity signals are altered, both bioelectrical and cytoskeletal patterns in the follicular epithelium change. We propose that not only cell-specific levels of V_memb and pH_i, or the polarities of transcellular electrochemical gradients, but also the slopes of these gradients are crucial for cytoskeletal modifications and, thus, for proper development of epithelial polarity.

Keywords: Drosophila melanogaster, Oogenesis, Electrochemical gradient, Follicle cell, Gurken, Pattern formation

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Background

Spatiotemporal electrochemical patterns affect cytoskeletal dynamics and play a role in defining spatial coordinates of tissues and organs in several species [1–8]. Therefore, it is tempting to investigate $V_{\text{mem}}$- and $\text{pH}_i$-gradients in relation to cytoskeletal patterns in a Drosophila mutant with disturbed axial polarity. Ovarian follicles of the mutant gurken (grk) show morphological defects concerning both the anteroposterior (a-p) and the dorsoventral (d-v) axis [9, 10]. In grk follicles, the oocyte nucleus (ON) is located at the posterior end of the oocyte (Ooc), and the follicular epithelium (FE) has a transversally uniform appearance (Fig. 1). Since ON movement to an anterodorsal position fails to occur in grk, both the longitudinal and the transversal axis are not correctly defined [11, 12]. In addition, Grk is required for border-cell (BC) migration to a position adjacent to the ON [13].

Grk, a transforming growth factor-α (TGF-α) homolog, is a ligand of the epidermal growth-factor receptor (EGFR) Torpedo (Top)/DER, and functions as a spatially restricted signal to activate the Egfr-pathway in follicle cells (FC) [14, 15]. Two rounds of Grk-Egfr signalling at different times during oogenesis generate axial polarity. In early oogenesis (stages S6–7), Egfr-activation in posterior FC (pFC) defines a-p polarity, whereas in mid-oogenesis (S9), restriction of Egfr-activity to dorsal FC determines d-v polarity [16]. Localised Grk-Egfr signalling depends on the position of the ON [9, 17, 18]. In strong grk mutants, both anterior and posterior FC adopt anterior fates, as indicated by the anterior-specific FC-marker slbo, and micropylar structures develop at

Fig. 1 Comparison of wt and grk follicles. a The dorsal side of wt S10B is defined by a thicker, columnar follicular epithelium (FE) and by an anterodorsal position of the oocyte nucleus (ON, red circle; cFC, centripetal follicle cells; mFC, mainbody follicle cells; pFC, posterior follicle cells). b grk S10B lacks dorsoventral (d-v) polarity and is characterised by a uniform cuboidal, ventralised FE covering the oocyte (Ooc). While, in wt S10B, border cells (BC) are located close to the ON, in grk S10B, disrupted body-axis formation leads to undefined positioning of BC amongst the nurse cells (NC). The grk ON is often located at the posterior end of the Ooc in a typical protrusion. c Transheterozygous combinations of grk alleles HF48 and 2B6 result in ventralised grk follicles of all vitellogenic stages (S8–14; bright-field image). In S12–14, wt-typical dorsal respiratory appendages are missing and a second micropylar structure appears at the posterior end. d To visualise basal microfilaments (bMF) and microtubules (MT) in the FE, tangential optical sections using structured-illumination microscopy (SIM; focal plane: red line) were used. For analysis of $V_{\text{mem}}$- and $\text{pH}_i$-patterns, median optical sections (SIM; focal plane: turquoise line) were used. e Quantification of transversal ($e_1$) and anteroposterior ($a$-$p$, $e_2$) gradients of $V_{\text{mem}}$ and $\text{pH}_i$, respectively, in the FE of S10B. Example of a grk follicle (SIM) where DiBAC-fluorescence intensities of FE1 (area marked in yellow) and FE2 (white) as well as of aFE (red) and pFE (blue) were measured using ImageJ (“mean grey value”). In wt follicles, the d-v axis was identified via the anterodorsal position of the ON, and the fluorescence intensities of the dorsal and ventral FE were quantified accordingly.
both poles [9, 17]. In the wild-type (wt), the pFC, in return, signal back to the Ooc, inducing a reorganisation of the microtubule (MT) cytoskeleton and leading to the MT-dependent migration of the ON to an anterior position [12, 19]. As a result, the overlying FC receive the Grk-signal and adopt dorsal fates [16, 20, 21]. This symmetry-breaking step is likely to be a prerequisite for the asymmetrical distribution or activation of ion-transport mechanisms in the FE observed later in development [4].

Participation of bioelectrical signals during axis formation has been demonstrated, e.g., for left-right patterning in *Xenopus* and chick embryos [22, 23], for lateral embryonic eye patterning in *Xenopus* [24], and for a-p patterning in planaria [25, 26]. In particular, the cytoskeleton is an attractive candidate for bioelectrical signalling, since binding of actin-associated factors [27, 28] as well as contractility of actomyosin complexes are controlled by pH i [29]. On the other hand, MT are known to amplify electrical signals [30, 31], and modifications of the cytoskeletal organisation have been shown to be promoted by changes in V mem [8, 32, 33].

For some *Drosophila* mutants with altered axial polarity, connections between morphological polarity and bioelectrical signals have already been described: For example, in egalitarian or Bicaudal-D mutant follicles, where no Ooc and no a-p or d-v polarity is established, aberrant patterns of extracellular ionic currents correlate with disturbed axial polarity [34–36]. In addition, in follicles of the mutant dicephalic, where NC appear at both ends of the Ooc, altered current patterns correlate with impaired a-p polarity [34, 35].

Previous studies on cytoskeletal functions in the FE of *Drosophila* have revealed the requirement of MT in posterior migration of BC (to the Ooc) and in centripetal migration of FC (between NC and Ooc) [37]. On the other hand, the organisation of microfilaments (MF) in the FE corresponds to FC differentiation and plays a decisive role in shaping the follicle along its longitudinal axis [38, 39]. It has also been shown that pH r- and V mem-changes induced by several inhibitors of ion-transport mechanisms located in the FE [7] simulate naturally occurring bioelectrical changes [4] and lead to alterations of MF- and MT-patterns as observed during FC differentiation [8]. Therefore, gradual modifications of electrochemical signals can serve as physiological means to regulate cell and tissue architecture by modifying cytoskeletal patterns [8].

In the present study, we compare wt and grk follicles with regard to their bioelectrical signals, using a fluorescent pH-indicator and a potentiometric dye [4, 7]. In addition, we compare wt and grk follicles with regard to their cytoskeletal organisation, using fluorescent phalloidin and an antibody against acetylated α-tubulin [8]. Since, in the wt FE, changes in cytoskeletal patterns are linked to changes in bioelectrical properties, it is tempting to analyse correlations between bioelectrical polarity, cytoskeletal polarity and axial polarity in the polarity mutant grk.

**Results**

**Bioelectrical differences between wt and grk**

Almost all follicles produced by transheterozygous grk females show morphological defects concerning both axes (Fig. 1). These grk follicles are characterised by a transversally uniform, cuboidal FE covering the Ooc, and by an ON located, predominantly, at the posterior end. This contrasts with wt follicles, where the dorsal side in S10B is defined by a thicker, columnar FE and an anterodorsal position of the ON [11]. These morphological peculiarities correlate with stage-specific differences between wt and grk concerning V mem- and pH r-patterns in the FE, as revealed by the potentiometric dye DiBAC and the pH-indicator CFDA, respectively (Fig. 2). As described earlier [4, 7], stronger fluorescence intensities refer to more depolarised V mem or more alkaline pH r, whereas weaker fluorescence intensities refer to more hyperpolarised V mem or more acidic pH r.

During early to mid-vitellogenic stages S8-10A, overall V mem- and pH r-patterns of wt (Fig. 2a-c and m-o) and grk (Fig. 2g-i and s-u) are rather similar, since d-v gradients have not yet emerged in the wt (cf. [4, 7]). In both wt and grk, the somatic FE in S8 is more depolarised and more acidic than the germline cells (Ooc and NC). During S9-10A, grk follicles develop a similar a-p V mem-pattern as wt follicles, the mainbody follicle cells (mFC) being hyperpolarised in relation to the neighbouring pFC and centripetal follicle cells (cFC). In addition, during S9-10A, a-p pH r-gradients are present in the FE of both wt and grk, the pFC being most alkaline. Additionally, in both genotypes, the anterior-most NC is the most alkaline. However, in the S9 FE of wt and grk, a closer look reveals that the slopes of the a-p V mem-gradients differ (Fig. 2b and h; for variability between follicles of the same stage, see Additional file: Fig. S1). Compared to wt, the mFC in grk are less hyperpolarised in relation to neighbouring cFC, and the whole FE is more depolarised, resulting in a shallower a-p V mem-gradient (significantly reduced angle of gradient; Table 1 and Fig. 3a).

Even more striking differences become obvious during S10B, when d-v electrochemical gradients are established in the FE of wt (Fig. 2d and p) but not grk (Fig. 2j and v; Table 2 and Fig. 3b); for variability between follicles of the same stage, see Additional file: Fig. S1). In most analysed wt S10B follicles, according to the position of the ON, the more depolarised or more alkaline side was identified as the ventral side (cf. [4, 7]). In some grk S10B follicles, a transversal V mem-gradient was detected...
but, in contrast to wt, such a gradient was absent from later stages (S11–12; Fig. 2e,f and k,l). A transversal pH$_i$ gradient, however, was never observed in grk (Fig. 2v–x; Table 2 and Fig. 3b; Additional file: Table S1). Concerning a–p electrochemical gradients, significant differences between wt and grk were not observed (Table 2 and Fig. 3c; Additional file: Table S2).

Using fluorescent phalloidin and an antibody against acetylated α-tubulin, we compared, during S8–12, the FE of wt and grk concerning cytoskeletal organisation (Figs. 4 and 5; for wt, cf. [8]). During S8, basal microfilaments (bMF) show the same parallel transversal alignment in wt and grk (Fig. 4a,g). Except for wt S10A (Fig. 4c), this alignment is missing in wt S9, S10B and S11,
but it persists during these stages in grk. In all cFC in wt S9, and in dorsal cFC in wt S10B, condensations of bMF appear (Fig. 5; cf. [8]). This phenomenon is accompanied by a loss of the transcellular parallel alignment of bMF in the remaining wt FE (Fig. 4b, d; for variability between follicles of the same stage, see Additional file: Fig. S2). In wt S11, a rearrangement of bMF occurs with fan-shaped structures (Fig. 4e; cf. [8]), and in wt S12, a dense transversal pattern of parallel bMF develops (Fig. 4f). In grk S11, many FC seem to contain no bMF (Fig. 4k). Moreover, in several grk S12 follicles, bMF were almost totally missing, while other follicles exhibit transversally aligned bMF (Fig. 4l). Thus, as observed for bioelectrical properties, the grk FE exhibits striking stage-specific peculiarities concerning the bMF-pattern. In contrast to wt, grk bMF are characterised by transversal alignment during all analysed stages, while wt-typical condensations in cFC (S9) and dorsal cFC (S10B) are absent (Fig. 4g-i; Fig. 5).

During S8, all wt FC show a more or less cell-autonomous organisation of MT, being arranged around the nuclei (Fig. 4m). Beginning with S9, the MT in wt cFC and mFC develop a longitudinal alignment, while the MT in pFC maintain their cell-autonomous arrangement (Fig. 4n). The longitudinal MT-alignment begins in cFC and spreads out over mFC to pFC (cf. [8]). However, in grk, in the whole MT alignment of MT was observed during all analysed stages (Fig. 4s-x). Between neighbouring FC, this MT-alignment in grk appears to be more coordinated than in wt. Thus, as observed for bMF, the MT pattern in the S9 FE of grk shows a more or less cell-autonomous organisation along the longitudinal axis (Fig. 4s-x).

The characteristic bioelectrical and cytoskeletal features of wt and grk in S9 and S10B are summarised in Fig. 6. In early vitellogenic stages (up to S9), a-p gradients of $V_{mem}$ and pH$_i$ show the same polarity in both genotypes. However, the a-p $V_{mem}$-gradient in grk S9 is shallower, and the whole FE is more depolarised compared to wt. In grk S9, bMF are characterised by transversal and MT by longitudinal alignment, whereas both wt-typical condensations of bMF and cell-autonomously organised MT are absent. During S10B, striking bioelectrical as well as cytoskeletal differences appear, when d-v polarity becomes obvious in wt but not in grk. In wt S10B, prominent a-p and d-v gradients of both $V_{mem}$ and pH$_i$ appear in combination with condensations of bMF in dorsal cFC and cell-autonomously organised MT in mFC and pFC. In grk S10B, however, d-v $V_{mem}$- and pH$_i$-gradients are missing and both condensations of bMF and cell-autonomously organised MT are absent (Fig. 6).

### Discussion

**Altered axial polarity correlates with altered electrochemical gradients**

At the posterior pole of grk follicles older than S9, migrating FC can be observed which, more or less, enclose the ON. According to previous reports [9, 17], the three anterior FC types (BC, stretched FC and cFC) are duplicated at the posterior end of grk follicles. The “posterior FC” in grk undergo similar morphological movements as the anterior FC. “Posterior BC” lose their epithelial organisation, “adjacent posterior FC” become stretched and “posterior cFC” migrate centripetally, sometimes even bisecting the Ooc. These aberrations of axial polarity in the FE of grk correlate with altered bioelectrical and cytoskeletal patterns as described in the present study.

In the FE of wt and grk, we compared stage-specific longitudinal and transversal gradients of $V_{mem}$ and pH$_i$, respectively. Since d-v electrochemical gradients are not yet established in the wt during early to mid-vitellogenic stages S8-10A (cf. [4, 7]), the overall $V_{mem}$- and pH$_i$-patterns of wt and grk are rather similar. However, corresponding to impaired a-p polarity in grk, the slope of the a-p $V_{mem}$-gradient in S9 is significantly reduced compared to wt.

More striking bioelectrical characteristics relating to missing d-v polarity in grk appear in S10B. During grk S10B-12, significant transversal electrochemical gradients are absent. In early wt S10B, the FE becomes continuously depolarised from dorsal to ventral while pH$_i$ increases in the same direction. During late wt S10B-12, dorsal cFC show increasing depolarisation (cf. [7]). Referring to morphological variability, some grk S10B follicles exhibit a transient transversal $V_{mem}$-gradient which was never observed during later stages.

**Altered electrochemical gradients correlate with altered cytoskeletal patterns**

As shown in detail recently [8], stage-specific alterations of $V_{mem}$ and pH$_i$ correlate with structural modifications of bMF and MT in the wt FE. Higher pH$_i$, as observed in mFC and pFC in S10B, stabilises the parallel

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**Table 1**

In the S9 FE of grk, the a-p $V_{mem}$-gradient is shallower.

| Gradient | $V_{mem}$ | pH$_i$ |
|----------|-----------|--------|
| a-p      | Fraction of S9 follicles | Fraction of S9 follicles |
| a-p with cFC/mFC $\geq 1.5^a$ | $\geq 1.3^a$ | $=5$ ; $2/5$ |
| wt       | 5/5       | 2/5    |
| grk      | 0/5       | 2/5    |

$^a$ a-p $V_{mem}$-gradients ($fluorescence$ $intensity$ $ratios$ cFC/mFC) in the S9 FE of wt and grk were evaluated as described previously [7]. $^a$ a-p pH$_i$-gradients ($fluorescence$ $intensity$ $ratios$ pFE/aFE) were quantified according to Fig. 1e2. The higher the fluorescence intensity ratio is, the steeper is the gradient ($n = 5$; see also Fig. 3a)
alignment of bMF and results in loss of the longitudinal alignment of MT (leading to a more cell-autonomous MT-arrangement). Lower pH$_i$, as observed in dorsal cFC in early S10B, leads to increasing disorder and condensation of bMF as well as to stabilisation of the longitudinal MT-alignment. Lower pH$_i$ in combination with relatively depolarised V$_{mem}$, as observed in dorsal cFC in late S10B, contributes to the disintegration of bMF. Correlations between bioelectrical properties and cytoskeletal patterns, as observed in different stages and different regions of the wt FE, correspond to correlations induced by inhibitors of various ion-transport mechanisms [8]. These observations lend support to the hypothesis that gradual modifications of electrochemical signals can serve as physiological means to regulate cell and tissue architecture by modifying cytoskeletal patterns [7, 8].

Further support to this hypothesis is provided by the present study. Shallower (or no) V$_{mem}$-gradients and
Table 2 In the S10B FE of grk, distinct transversal $V_{mem}$- and pH$_i$-gradients are missing

| Gradients         | Fraction of S10B follicles | Fraction of S10B follicles |
|-------------------|-----------------------------|-----------------------------|
| transversal$^b$   | with FE$_2$/FE$_1$ ≥ 1.5    | with FE$_2$/FE$_1$ ≥ 1.5    |
| wt                | 5/7                         | 4/7                         |
| grk               | 1/7                         | 0/7                         |
| a-p$^a$           | with pFE/aFE ≥ 1.5          | with pFE/aFE ≥ 1.5          |
| wt                | 2/7                         | 4/7                         |
| grk               | 4/7                         | 6/7                         |

$^a$Transversal gradients (fluorescence intensity ratios FE$_2$/FE$_1$; larger value vs. smaller value), and $^b$ a-p gradients (fluorescence intensity ratios pFE/aFE) in the S10B FE of wt and grk were quantified as shown in Fig. 1e (for variability see Additional file: Tables S1 and S2). The higher the fluorescence intensity ratio is, the steeper is the gradient (n = 7; see als Fig. 3b and c). Since, in grk, the cFC and mFC (referring to aFE) are often both more hyperpolarised and more acidic than the aberrant pFC, a-p gradients in grk seem to be somewhat steeper than in wt (cf. Additional file: Fig. S1 and Table S2), but this difference was not significant (cf. Fig. 3c).

relative alkalisation, as generated by the inhibition of certain ion-transport mechanisms [7], lead to stabilisation of the parallel transversal bMF-pattern in wt [8]. This also holds true for the cFC in grk S9 and the dorsal cFC in grk S10B, where bMF retain their transversal alignment while wt-typical condensation and subsequent disintegration of bMF are missing. Similarly, shallower (or no) $V_{mem}$-gradients lead to stabilisation of the longitudinal MT-orientation in wt [8]. This also holds true for mFC and pFC in grk S9 as well as in grk S10B, where, in addition, a transversal pH$_i$-gradient is missing. In the whole grk FE, MT exhibit a longitudinal transcellular alignment, whereas in wt mFC and pFC, MT-patterns are characterised by a more cell-autonomous organisation.

In grk, preferential alignment of bMF along the transversal axis and of MT along the longitudinal axis is obviously enhanced. Assuming a duplication of anterior FC types at the “posterior pole” in grk [9, 14, 17], it seems plausible that both the transversal alignment of bMF and the longitudinal alignment of MT, as found in the anterior FE of grk, is duplicated in the “posterior FE”.

In wt S9, longitudinal electrochemical gradients with relative depolarisation and relative acidification in cFC result in condensation of bMF and in longitudinally aligned MT in this area [8]. Impaired a-p polarity in grk S9, however, leads to relative depolarisation in the whole FE resulting in a shallower longitudinal $V_{mem}$-gradient and in stabilisation of the transversal bMF-pattern. In wt S10B, strong transversal electrochemical gradients, showing relative hyperpolarisation and relative acidification in dorsal cFC, lead to condensation and disintegration of bMF [8]. On the other hand, as a consequence of missing d-v polarity in grk S10B, transversal electrochemical gradients as well as bMF-condensation and disintegration are absent from the whole FE.

Therefore, we propose that shallow or missing electrochemical gradients, as observed in grk, result in stabilisation of cytoskeletal patterns. Throughout oogenesis, the bMF remain oriented along the transversal axis while the MT remain oriented along the longitudinal axis. This interpretation corresponds to the previous observation that experimentally reduced $V_{mem}$-gradients stabilise both bMF- and MT-patterns [8].

**Conclusion**

Our analysis of the *Drosophila* mutant grk leads to the conclusion that not only cell-specific levels of $V_{mem}$ and pH$_i$, or the polarities of electrochemical gradients [8], but also the slopes of these gradients are crucial for either alteration or stability of cytoskeletal patterns. When primary signals of axial polarity, like Grk, are weak or missing, ion-transport mechanisms and gap junctions in the FE are not distributed or activated asymmetrically. Consequently, electrochemical gradients are shallow and patterns of cytoskeletal elements remain unchanged.

**Methods**

**Fly stocks**

For analysis, *Drosophila melanogaster* wild-type Oregon R (wt) and *gurken* (grk) were used. The strains *w; grk*$_{HF48}$/CyO and *w; grk*$_{2B6}$/CyO (gift of S. Roth and O. Karst, Köln, Germany) were crossed to generate transheterozygous *grk*$_{HF48}$/*grk*$_{2B6}$ flies. Although *grk*$_{2B6}$ is the strongest existing allele [14, 45, 46], only a combination of both grk null alleles led to a penetrance of 100% ventralised grk follicles (Fig. 1c). Flies were reared at 25 °C in the dark on standard food with additional fresh yeast.

**Preparation of follicles**

Females were killed by crushing the head with tweezers without anaesthesia, and 3 days old wt or 2 days old grk ovaries were dissected (older grk ovaries contained many degenerating follicles). Single follicles of stages S8–12 were isolated from the epithelial sheath by pulling at the anterior tip of an ovariole. Dissection was carried out in...
**Drosophila** phosphate-buffered saline [47]. For staining with fluorescent indicators, we used R-14 Medium [47] which is best suited for in-vitro culture of **Drosophila** follicles [48].

**Fluorescent membrane potential indicator**

For the analysis of $V_{m}$-patterns, we used the fluorescent potentiometric dye DiBAC (DiBAC$_{4}$(3); bis-(1,3-dibutylbarbituric acid) trimethine oxonol; Molecular Probes/Thermo Fisher Scientific, USA). As described earlier [4, 7], relative depolarisation leads to intracellular accumulation of the anionic dye and to increasing fluorescence while relative hyperpolarisation leads to decreasing fluorescence. Living follicles were incubated for 20 min in R-14 medium containing 4 $\mu$M DiBAC (dissolved in 70% ethanol). Thereafter, they were mounted in R-14 medium and analysed immediately using $\times$10/0.25 and $\times$20/0.5 objectives and median optical sections (Fig. 1d) on a Zeiss AxioImager.M2 structured-illumination microscope (SIM), equipped with a Zeiss ApoTome, a
Zeiss AxioCamMRm camera and the appropriate filter set. For numbers of analysed S8–12 follicles, see Additional file: Table S3.

Fluorescent intracellular pH indicator
For the analysis of pH-i-patterns, we used the fluorescent pH-indicator CFDA (5-CFDA,AM; 5-carboxyfluorescein diacetate, acetoxymethyl ester; Molecular Probes) which enters cells as an anion. As described earlier [4, 7], increasing fluorescence indicates relative alkalisation while decreasing fluorescence due to protonation indicates relative acidification. Living follicles were incubated for 20 min in R-14 medium containing 4 μM CFDA (dissolved in dimethyl sulfoxide, DMSO). Subsequently, the follicles were mounted in R-14 medium and viewed immediately as described above using median optical sections (Fig. 1d). For numbers of analysed S8–12 follicles, see Additional file: Table S3.

F-actin staining using fluorescent phalloidin
Follicles were fixed in microfilament-stabilising buffer (MF-buffer [8, 38]) with 4% formaldehyde and 0.2% Triton X-100 for 20 min at room temperature, washed with phosphate-buffered saline (PBS) and stained with 0.25 μg/ml phalloidin-FluoProbes 550A (Interchim, France; dissolved in DMSO) in PBS. After washing, the follicles were mounted in Fluoromount G (Interchim) and viewed as described above using a × 40/1.3 oil objective and tangential optical sections (Fig. 1d). For numbers of analysed S8–12 follicles, see Additional file: Table S3.

Indirect immunofluorescence staining of microtubules
Follicles were fixed for 20 min at room temperature in MF-buffer as described above, washed with PBS containing 0.1% Triton X-100 and blocked for 1 h at room temperature with 2% bovine serum albumin (BSA)/0.1% Triton X-100 in PBS. Thereafter, the follicles were incubated overnight at 4 °C or for 1 h at room temperature in PBS containing 1% BSA/0.1% Triton X-100 and a monoclonal antibody against acetylated α-tubulin (6-11B-1; Santa Cruz Biotechnology, USA) diluted 1:100 [8]. After washing, the follicles were treated for 1 h at room temperature with goat anti-mouse-biotin (Dianova, Germany) diluted 1:200 in PBS containing 1% BSA/0.1% Triton X-100. Washing was repeated before the follicles were incubated for 30 min with streptavidin-TexasRed (Dianova) diluted 1:100 in PBS containing 1% BSA/0.1% Triton X-100. After washing, the follicles were mounted and analysed as described above using tangential optical sections (Fig. 1d). For numbers of analysed S8–12 follicles, see Additional file: Table S3. Controls were performed without primary antibody.

Staging of follicles and determination of axes
Follicles were staged according to criteria described previously [11, 49]. To determine the a-p axis, the anterior position of the NC was used as marker, while for the d-v axis, the anterodorsal position of the ON and the columnar dorsal FE (S10B) were used. For grk follicles, the same criteria for staging and axis determination were applied. Due to the posterior location of the ON and the
transversally homogeneous FE, no dorsal side was detectable in grk (Fig. 1).

Quantification of fluorescence intensities in the FE
To quantify both the longitudinal and the transversal V_{mem} and pH_{i}-gradients in the FE of grk and wt, respectively, we used median optical sections (Fig. 1d) of stained follicles. Fluorescence intensities (“mean grey value”) of both sides (FE1 and FE2 or aFE and pFE) were measured using ImageJ (Fig. 1e) and a ratio of both values was determined. For a-p V_{mem}-gradients in S9, fluorescence intensities of cFC, mFC and pFC were measured separately and ratios determined according to [7].

Fig. 6 Summary of prominent bioelectrical and cytoskeletal differences between the FE of wt and grk. In early vitellogenic stages, as for example S9 (a), patterns of V_{mem} (white to blue gradient) and pH_{i} (white to red gradient) in grk are similar to the respective patterns in wt. White refers to stronger fluorescence intensities, corresponding to relative depolarisation or relative alkalisation, while blue (V_{mem}) and red (pH_{i}) refer to weaker fluorescence intensities, corresponding to relative hyperpolarisation (blue) or relative acidification (red). Triangles indicate fluorescence-intensity gradients. In S9, both wt and grk show a-p V_{mem}-gradients, with mFC being hyperpolarised (blue) in relation to neighbouring cFC and pFC (white). The same holds true for a-p pH_{i}-gradients, with pFC being the most alkaline FC (white). However, the a-p V_{mem}-gradient in grk is shallower, since mFC are less hyperpolarised relative to neighbouring FC, and the whole FE is more depolarised compared to wt. In S10B (b), in relation to other FC, grk cFC show both slight hyperpolarisation and slight acidification, while dorsal wt cFC show strong hyperpolarisation as well as strong acidification. In both wt and grk, the BC exhibit relatively depolarised V_{mem} and relatively acidic pH_{i}. Concerning cytoskeletal organisation in S9 (a), grk bMF are characterised by transversal alignment (orange dashes) and grk MT by longitudinal alignment (green lines), while wt-typical bMF-condensations (orange asterisks) and cell-autonomously organised MT (green circles) are absent. From S10B onward (b), prominent d-v gradients of both V_{mem} and pH_{i} appear in the FE of wt, but not grk. This corresponds with peculiarities in both the bMF- and the MT-organisation: In the wt FE, condensations of bMF (orange asterisks) appear in dorsal cFC, and cell-autonomously organised MT (green circles) appear in mFC and pFC. In contrast, in grk, both condensations of bMF and cell-autonomously organised MT are absent from the whole FE.
Abbreviations
5-CFDA:AM; 5-Carboxyfluorescein diacetate, acetoxymethyl ester; aFE: Anterior half of the Fe; a-p: Anteroposterior; BC: Border cells; bMF: Basal microfilaments; BSA: Bovine serum albumine; cFC: Centripetal follicle cells; DiBAC4(3): Bis-(1,3-dibutylbarbituric acid) trimethine oxonol; DMSO: Dimethyl sulfoxide; d-v: Dorsoventral; FF: Follicle cells; FE: Follicular epithelium; gurk: gurken; MF: Microfilaments; mFC: Mainbody follicle cells; MT: Microtubules; NC: Nurse cells; O: Oocyte nucleus; Ooc: Oocyte; PBS: Phosphate-buffered saline; pFC: Posterior follicle cells; pFE: Posterior half of the Fe; pH: Intracellular pH; S: Stage; SIM: Structured-illumination microscopy; vmean: Membrane potential; wt: Wild-type

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Authors' contributions
SS carried out the experiments and analysed the data under the supervision of JB. JB conceived the study and reviewed the data. Both authors wrote the manuscript and read and approved the final version.

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Availability of data and materials
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