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Research

Cell wall-mediated maternal control of apical–basal patterning of the kelp *Undaria pinnatifida*

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Summary

- The role of maternal tissue in embryogenesis remains enigmatic in many complex organisms. Here, we investigate the contribution of maternal tissue to apical–basal patterning in the kelp embryo.
- Focussing on *Undaria pinnatifida*, we studied the effects of detachment from the maternal tissue using microsurgery, staining of cell wall modifications, morphometric measurements, flow cytometry, genotyping and a modified kelp fertilisation protocol synchronising kelp embryogenesis.
- Detached embryos are rounder and often show aberrant morphologies. When a part of the oogonial cell wall remains attached to the zygote, the apical–basal patterning is rescued. Furthermore, the absence of contact with maternal tissue increases parthenogenesis, highlighting the critical role of maternal signals in the initial stages of development.
- These results show a key role for the connection to the maternal oogonial cell wall in apical–basal patterning in kelps. This observation is reminiscent of another brown alga, *Fucus*, where the cell wall directs the cell fate. Our findings suggest a conserved mechanism across phylogenetically distant oogamous lineages, where localised secretion of sulphated F2 fucans mediates the establishment of the apical–basal polarity. In this model, the maternal oogonial cell wall mediates basal cell fate determination by providing an extrinsic patterning cue to the future kelp embryo.

Introduction

In many complex multicellular organisms, such as animals, red algae and land plants, zygotes develop in close association with the maternal tissue (Kölle *et al.*, 2020; Borg *et al.*, 2023; Woudenberg *et al.*, 2024). In plants, embryo development typically begins by establishing apical–basal polarity and an initial asymmetric division of the zygote which is embedded within maternal tissue. The extent of guidance, protection and developmental robustness that the maternal tissue can provide to plant embryogenesis remains an open question (Woudenberg *et al.*, 2024).

Contrary to land plants and red algae, most brown algal species release their gametes into the environment. Zygotes, therefore, develop free from maternal tissue. Additionally, the development of the zygotes may occur naturally synchronised by simultaneous gamete release and fertilisation. Consequently, studies on brown algae have offered important insights into cell polarisation and embryonic patterning in a lineage that evolved multicellularity independently from plants (reviewed in De Smet & Beeckman, 2011; Coelho & Cock, 2020; Bogaert *et al.*, 2023). Specifically, research on the brown alga *Fucus* has highlighted the critical role of cell fate determinants present in the extracellular matrix (ECM; Berger *et al.*, 1994; Bouget *et al.*, 1998).

The role of maternal tissue in the early development of brown seaweeds displays considerable variation. In *Fucus*, eggs are released in the environment and zygotes undergo polarisation entirely *de novo*, either in response to light or the entry of paternal sperm (Hable & Kropf, 2000). Conversely, *Dictyota* zygotes retain a maternally determined direction for the polarisation vector, while the sense of the vector is determined by the direction of light (Bogaert *et al.*, 2017a,b). In oogamous brown algae of the order Laminariales and family Phyllariaceae (Tilopteridales; here collectively referred to as 'kelps'), the release of female gametes is incomplete. Eggs and embryos remain physically

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connected to the female gametophyte after extrusion from the oogonium. However, relatively little is known about the relevance of this connection to the maternal tissue in apical–basal patterning.

The study of kelp embryogenesis is further conflated by the occurrence of parthenogenetic development (tom Dieck, 1992; Druehl *et al.*, 2005), with eggs being able to develop in the absence of fertilisation. A round morphology and a tendency to easily detach are two properties that are used to discern parthenotes from embryos during crossing experiments (tom Dieck, 1992; Martins *et al.*, 2017; Liboureau *et al.*, 2024). Parthenogenesis is marked by a higher likelihood of detachment (Martins *et al.*, 2017; Klochkova *et al.*, 2019), reduced growth, and various defects in mitosis and polarity leading to rounder embryos (Screiber, 1930; Fang *et al.*, 1979; tom Dieck, 1992; Ar Gall *et al.*, 1996).

In kelp, the eggs bear flagella, similar to the male gametes (Motomura & Sakai, 1988; Motomura, 1990). Only recently, it was shown that these female flagella anchor the egg to the oogonium (Klochkova *et al.*, 2019). The occurrence of flagella, basal bodies, flagellar roots and a polarised cytoplasm (Motomura, 1994) imply that the egg is a polarised cell. Exploring the fate of detached progeny in ecology is crucial for understanding dispersal mechanisms and adaptability of kelp populations to environmental changes.

Here, we used the kelp *Undaria pinnatifida* to test whether apical–basal polarity in kelp embryos is established entirely autonomously or whether signalling from the maternal gametophytic tissue is necessary for correct patterning and early embryonic development. In order to exclude confusion with putative polarity defects of parthenotes, we investigate the events underlying early embryogenesis and contrast these with parthenogenesis.

We show that kelp embryos lacking maternal contact lose their apical–basal polarity, leading to defects in developmental patterning which resemble those observed in parthenosporophytes. Using time-lapse imaging and micro-dissection, we can distinguish between the impacts of the cell wall and cytoplasmic signals from the maternal tissue. Our results show that the oogonial cell wall is required for apical–basal patterning of the embryo. Additionally, Golgi-derived cell wall material is deposited in the oogonium, reminiscent of another brown alga, *Fucus*, where the cell wall has been shown to play a key role in cell fate determination. We propose that factor(s) secreted into the oogonial cell wall by the maternal gametophyte determine the fate of the basal cell in the developing embryo, contributing to the developmental patterning robustness of the diploid sporophyte.

Materials and Methods

Algal strains and growth conditions

Experiments were carried out using uni-algal laboratory cultures of respectively *Undaria pinnatifida* (Harvey) Suringar (Upin_NL1f (female) and Upin_NL1m (male)), isolated from a single sporophyte sampled in Westerschelde (2020, the Netherlands). Additional observations were made on *Saccorhiza polyschides (Lightfoot) Batters* (PH-IS_016f (female) and PH-IS_001m (male)) (Perharidy, France), *Macrocystis pyrifera* (Linnaeus) C. Agardh (CVe13f (female) and CVe30m (male)) (2006, Curaco de Vélez, Chile) and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (Slat_FR1f (female), Slat_FR1m (male)) (2020, Brittany, France). For *Undaria*, fertility was induced by fragmenting male and female gametophytes. *Undaria* cultures were grown separately at 18°C at 80 µmol photons m^{-2} s^{-1} (Cool White; Lumilux, Osram, Germany) and a 12 h : 12 h, light : dark photoperiod in filtered and autoclaved natural seawater enriched with modified Provasoli (mPES; West & McBride, 1999). Fertility of *Macrocystis* and *Saccorhiza* was induced at 12°C under a 16 h : 8 h, light : dark photoperiod with 20–30 µmol photons m^{-2} s^{-1}. Fertility for *Saccharina* was induced at 10°C under 12 h : 12 h photoperiod at 10 µmol photons m^{-2} s^{-1}.

Synchronised fertility

To avoid that our control populations contain a mixture of age cohorts released at different days, it is important to control the timing of fertilisation. Therefore we designed a protocol for synchronised kelp fertilisation, making use of two key features of kelp reproduction: in a light–dark cycle, female gametophytes mainly release eggs within 30 min of darkness (Lüning, 1981), and a pheromone produced by the released eggs triggers sperm release and attraction (Maier *et al.*, 2001). A filtered medium of female gametophytes containing freshly released eggs collected after 2 h of darkness was added to fertile male gametophytes to trigger sperm release. Two minutes after the induction, the male medium containing suspended sperm cells was filtered through a sterile 40-µm nylon mesh to remove the male gametophytes. The sieved suspension with male gametes was combined with the remaining female gametophyte culture to induce fertilisation, followed by incubation in darkness to continue the night cycle. This method ensures the production of a single age cohort of sporophytes by allowing only a short time window for fertilisation.

Detachment of zygotes

Fertilised suspended female gametophytes were rinsed over a 40-µm nylon mesh to remove loose eggs and parthenotes and zygotes. Fertile gametophytes were pipetted up and down using a Pasteur pipette for 3 min to detach zygotes. The culture was then filtered again through a 40-µm nylon mesh, and detached zygotes and eggs were isolated in a 24-well plate.

Parthenogenesis control

For describing and controlling for the effects of parthenogenetic development, we detached unfertilised eggs in female-only cultures. Eggs were detached as described for zygotic development. Parthenogenesis was scored as development beyond a single-cell state at Day 4 after detachment.

Microsurgery in the oogonial cell wall

Using a Sharpoint 4-mm microsurgical knife, a precise incision was made between the live gametophyte and the *Undaria* zygote, leaving a piece of the oogonial cell wall attached to the zygote. After 4 d of cultivation, growth was assessed. In *Saccharina*, detachment of zygotes by pipetting resulted in a mixture of putative zygotes, with some retaining an attached piece of the oogonial cell wall, while others showed no visible remainder of the cell wall. Therefore, microsurgery as described for *Undaria* was not necessary.

Microscopy

Embryos were photographed at Days 1 and 4 with a Zeiss Axioplan 2 light microscope (Carl Zeiss, Jena, Germany) equipped with an AxioCam camera and an Olympus BX-51 microscope (Olympus, Tokyo, Japan) equipped with a digital camera (ToupCamTM, Touptek, China). ImageJ was used to measure several shape characteristics, including area, circularity, roundness and aspect ratio (AR; Schneider *et al.*, 2012). Both roundness and circularity values range from 0 to 1, with higher values indicating shapes that closely resemble a perfect circle. Lower circularity values correspond to more irregular shapes, while AR ranges from 1 to positive infinity and describes its elongation or flattening by comparing the major axis to the minor axis.

Statistics

For comparing two independent groups, when the data were normally distributed and variances were equal as confirmed by Levene's test, a Student's *t*-test was applied. In cases where the data did not meet these assumptions, a nonparametric Wilcoxon rank-sum test was employed. To examine the proportion of embryos to the total count of released eggs in different groups, a generalised linear model (GLM) with a quasi-binomial distribution was utilised to account for the overdispersion in the data. *Post hoc* comparisons between group levels were conducted using estimated marginal means with Tukey's adjustment for multiple comparisons to control the family-wise error rate. For comparing the morphometric measurements between groups, controlling for embryo size, a GLM with a Gamma distribution and log link function was used (for AR) or a beta regression model (Cribari-Neto & Zeileis, 2010) with logit link function for circularity and roundness which ranges between 0 and 1 to account for heteroscedasticity. Analyses were conducted in R (v.4.1.2; R Core Team, 2023).

Genotyping of embryos and parthenotes

To ascertain that *Undaria* embryos resulted from fertilised eggs, we amplified sex markers M_68_16_2 (length *c.* 350 bp) and M_285_20_2 (length *c.* 600 bp) (Lipinska *et al.*, 2015) using the KAPA3G Plant PCR Kit for direct genotyping and compared with molecular weight markers SmartLadder (200–10 000 base pairs) or SmartLadder SF (100–1000 base pairs) (Eurogentec, Seraing, Belgium). Samples were isolated at 4 d in PCR-grade water, crushed and stored at −20°C. Polymerase chain reactions were conducted with the following polymerase chain reaction conditions: initial: 95°C for 5 min; 40 cycles: 95°C (20 s), 65°C (25 s) and 72°C (30 s); and final: 72°C (7 min).

Flow cytometry

For the ploidy analysis, small clusters of gametophytes weighing 20–50 mg were dried lightly, finely chopped in 500 µl of nuclei isolation buffer and treated with 0.5 µg l^{-1} Proteinase K, 5‰ SDS and 0.1 mM Tris HCl at pH 8.0. The nuclei were then filtered through a 10-µm nylon mesh and dyed with 1× SYBR Green I. Following a 10-min incubation, the DNA content was analysed using an Agilent NovoCyte Advanteon flow cytometer.

Toluidine blue stainings

Cell wall modification in the oogonial cell wall was assayed by toluidine blue O (TBO) staining, which stains sulphated fucans indicative of polar secretion of Golgi-derived material into the cell wall in *Fucus* (Shaw & Quatrano, 1996). Gametophytes were stained for 15 min with 0.1% TBO artificial seawater at pH 1.5. Slides were rinsed three times in 99% ethanol for *c.* 5 min and once for 1 h before being mounted in tap water and photographed.

Results

Establishment of apical–basal polarity during early development

To describe the timing of the expression of the apical–basal polarisation vector, we investigated early embryogenesis in *Undaria*. When female gametophytes reach maturity, eggs are extruded from the oogonium, but remain connected to the oogonial aperture through two flagella (Fig. 1a,b). Initially spherical, *Undaria* eggs elongate within the first hour after fertilisation (AF) by expansion of the cell volume, and not by mere shape change (Fig. 1a,c, Supporting Information Figs S1, S2). The basal part of the zygote bulges and this bulge serves to anchor the embryo to the opening of the oogonial cell wall (Figs 1, S1). At *c.* 15 h AF, a clear hyaline zone at the site of the future cytokinesis plane becomes evident and zygotes undergo a first, asymmetric cell division (Figs 1d, S1). The first cell division results in the formation of an apical cell, which will develop into the upper part of the thallus, and a basal cell, which will develop into the lower part of the thallus and secure the embryo to the oogonial cell wall of the gametophyte (Fig. 1a).

In order to understand how the apical–basal axis is expressed in normal embryo development, we described development following the first cell division. Zygotes typically divide twice transversely, that is perpendicular to the polarisation vector, resulting in two cells derived from the basal cell and two cells derived from the apical cell (Fig. 2a–c). The three most basal cells divide transversely again, while the most apical cell divides parallel to the

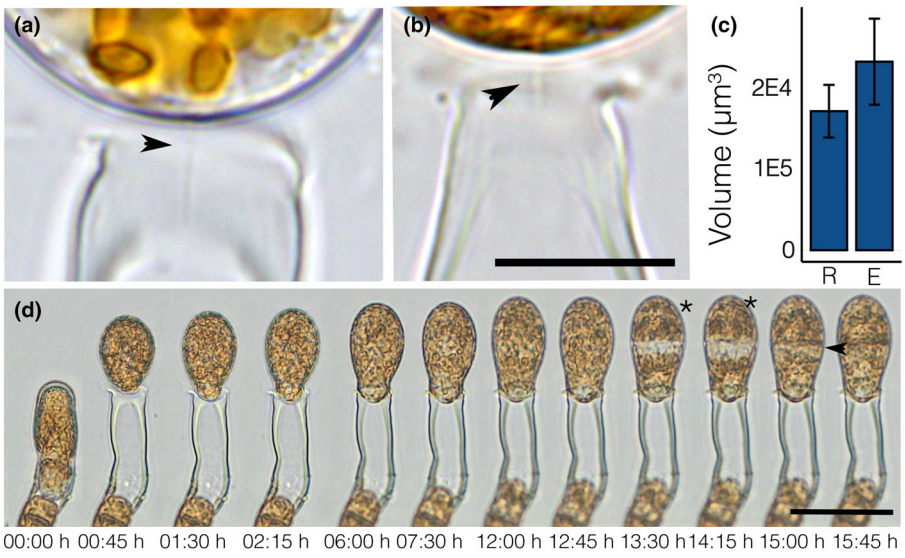

Fig. 1 Connection to the oogonium, elongation and first cell division of the *Undaria pinnatifida* zygote. (a, b) The embryo is connected to the oogonial cell wall via anchor flagella (arrowhead). Bar, 5 µm (c) Volume (±SD) increase between round cells (R) and elongated cells (E) (P = 0.0006, Wilcoxon rank-sum exact test). (d) Time lapse of egg release, elongation and first asymmetric cell division in the zygote. The first cell division is visible as a hyaline zone (*) before the completion of the cell plate. Apical and basal cells have a different fate, and the basal cell forms a basal bulge firmly attaching the zygote to the oogonial cell wall. Bar, 50 µm.

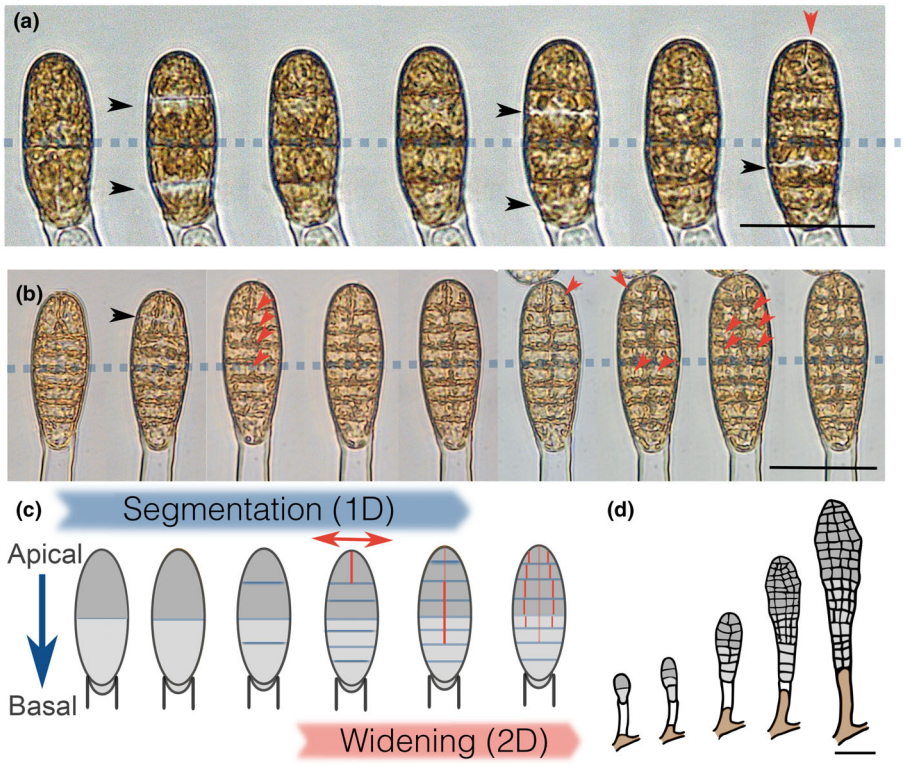

Fig. 2 Early development of the *Undaria pinnatifida* embryo. (a, b) Both apical and basal cells of the embryo first divide perpendicular (black arrowheads) to the long axis of the embryo after which cell division changes orientation parallel (red arrowheads) to the polarisation axis in the most apical cell. The three most basal cells divide perpendicular again, after which more parallel cell division occurs. Blue dashed line separates the apical from the basal cell line. (c) Schematic representation of the orientation of first cell and apical–basal gradient in cell division. Blue, transversal cell divisions perpendicular on the apical–basal vector (blue arrow); red cell divisions, parallel to the apical–basal vector (red arrow). (d) Schematic representation of blade expansion at apical end and female gametophyte expanding into the oogonial tube with new tissue. Drawings are based on time-lapse pictures taken at Days 0, 1, 2, 3 and 4. Bar, 50 µm.

apical–basal axis, initiating the process of blade widening (Fig. 2c). The resulting two apical cells divide transversely resulting in a total of eight segments. These segments further subdivide predominantly parallel to the polarisation vector in the apical half. The most basal cells divide substantially less (Fig. 2b,c). Further growth results in an elongated, one-layered blade with clear apical–basal polarity by Day 4 AF (Fig. 2d). At the same time, remaining vegetative cells of the female gametophyte undergo tip growth and gradually fill the empty space created by extrusion of the egg. The gametophyte ultimately will make contact with the embryo at *c.* Day 3 or 4, when the embryo is measuring 40 cells or more (Figs 2d, S3). Therefore, embryos develop in direct contact only with the oogonial cell wall for the first days.

Embryos and parthenotes exhibit distinct developmental trajectories

In kelps, eggs may initiate parthenogenetic development in absence of fertilisation (Shan *et al.*, 2013). These parthenotes tend to grow rounder (Ar Gall *et al.*, 1996; Fang *et al.*, 1979), detach more easily (Martins *et al.*, 2017; Klochkova *et al.*, 2019)

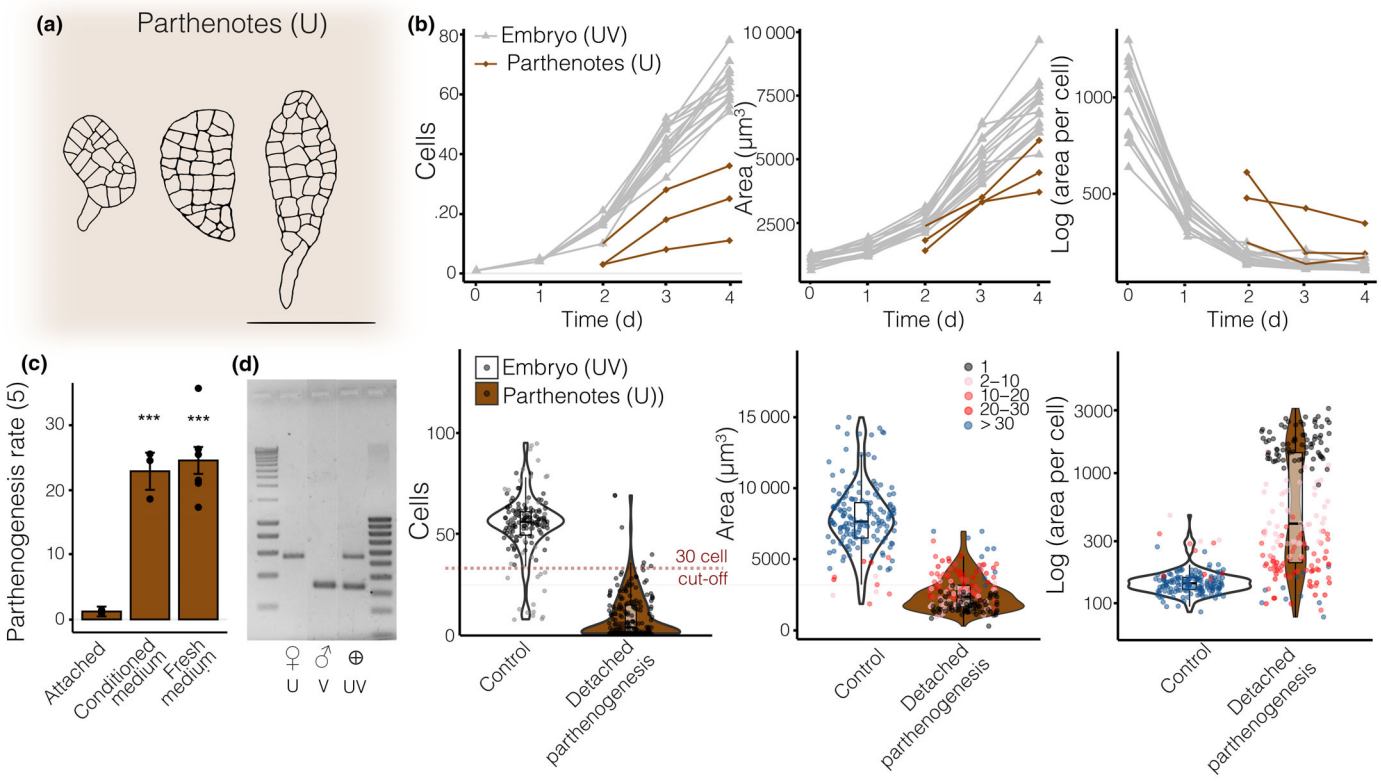

Fig. 3 Parthenogenesis can be recognised by a slower growth and cell division in *Undaria pinnatifida*. (a) Representative morphology of parthenotes at Day 4. Bar, 50 µm. (b) Upper panels, patterns of cell division, area and average cell size of representative parthenotes (red) vs embryos (grey) in the first 4 d of development. Lower panels, cell numbers, area and average cell sizes of embryos and detached parthenotes at 4 d after fertilisation (embryos) or detachment (parthenotes). Red dashed line denotes 30-cell cut-off used to filter out the bulk of the parthenotes. The number of cells in parthenotes and embryos at Day 4 is represented by colour: black, 1-celled; red, < 30 cells; and blue > 30 cells. (c) Parthenogenesis rate (±SE) is significantly increased regardless whether fresh or female conditioned medium was applied (*post hoc* pairwise comparisons; ***, *P*-value < 0.001). (d) Representative lanes from a gel electrophoresis illustrating sex markers discriminate between female gametophytes or parthenotes (U), male gametophytes (V) and sporophytes (UV) compared with molecular weight markers SmartLadder (200–10 000 bp, left) and SmartLadder SF (100–1000 bp, right).

and consequently an enrichment of parthenogenetic (rounder) individuals in the detached fraction may be expected. Moreover, patterning defects are a sign of parthenogenesis (tom Dieck, 1992; Martins *et al.*, 2017; Liboureau *et al.*, 2024). Therefore, in this study, we ensured that any patterning defects are not due to the effects of parthenogenesis. For this reason, we first had to examine the developmental patterns of parthenogenesis to discover how to discern parthenotes from embryos. We examined the developmental patterns of parthenotes in order to distinguish these from proper embryo development and the effect of maternal tissue on apical–basal patterning (Fig. 3a,b). To do so, female gametophytes were cultivated in the absence of males for 7 d until they reached fertility and produced eggs. We then scored the presence of parthenotes. We found that parthenogenesis events are rare (1.2% ± 0.7%) compared with control populations where eggs remain attached to the maternal gametophyte. We then tested the effect of detaching the eggs and cultivating them in natural SW or in a gametophyte-conditioned medium. The proportion of parthenogenetic embryos dramatically increased when the eggs were experimentally detached from the maternal gametophyte (Fig. 3c), regardless of whether gametophyte-conditioned medium or fresh medium was applied to the detached eggs. Therefore, we could not control parthenogenesis and relied on the difference in growth kinetics and polymerase chain reaction-based sex markers to discriminate embryos from parthenotes.

We compared the growth kinetics of female parthenotes and embryos over 4 d (Fig. 3b). Parthenotes exhibited a slower rate of cell division compared with embryos, rarely reaching the 30-cell state at Day 4. By contrast, most embryos reached > 50 cells at Day 4 (t = 31.058, df = 296.68, P-value < 2.2e−16; Welch two-sample t-test, Fig. 3c). Embryos were also significantly larger than parthenotes (t = 25.273, df = 229.33, P-value < 2.2e−16; Welch two-sample t-test). The reduced size of parthenotes was due to a delay in the onset of the first cell divisions (Fig. 3b). Indeed, while embryos showed a decrease in cell size during the first 2 d due to segmentation of the large zygote into smaller cells, in many of the parthenotes cell division did not keep up with growth, resulting in larger cells. Cell sizes in parthenotes were typically larger but showed an increased variance, reflecting a developmental delay. Only a small fraction (8%) of parthenotes reached the 30-cell stage by Day 4 (Fig. 3b). Thus, the bulk of parthenotes can be clearly differentiated from embryos based on the cell number at Day 4.

In *Ectocarpus*, parthenogenesis of unfertilised female gametes involves endoreduplication (Lewis *et al.*, 1993; Bothwell *et al.*, 2010). We found no evidence for endoreduplication in any of the analysed *Undaria* parthenotes (Fig. S4). In order to further differentiate embryos from parthenotes, we used sex-specific polymerase chain reaction primers to genotype the detached embryos (Fig. 3d). This allowed us to confirm the presence of male and female markers in detached embryos and exclude any potential enrichment in parthenogenetic embryos in our detached fraction.

Together, our results indicate that detachment from the maternal gametophyte induces parthenogenetic development that cannot be repressed by the presence of a gametophyte-conditioned medium. Parthenogenesis may be a confounding factor during analysis of early development in *Undaria*, but by assessing the number of cells at 4 d after germination and using polymerase chain reaction-based sex markers (Fig. 4), we could effectively discriminate embryos, containing both markers, from parthenotes, containing only the female marker.

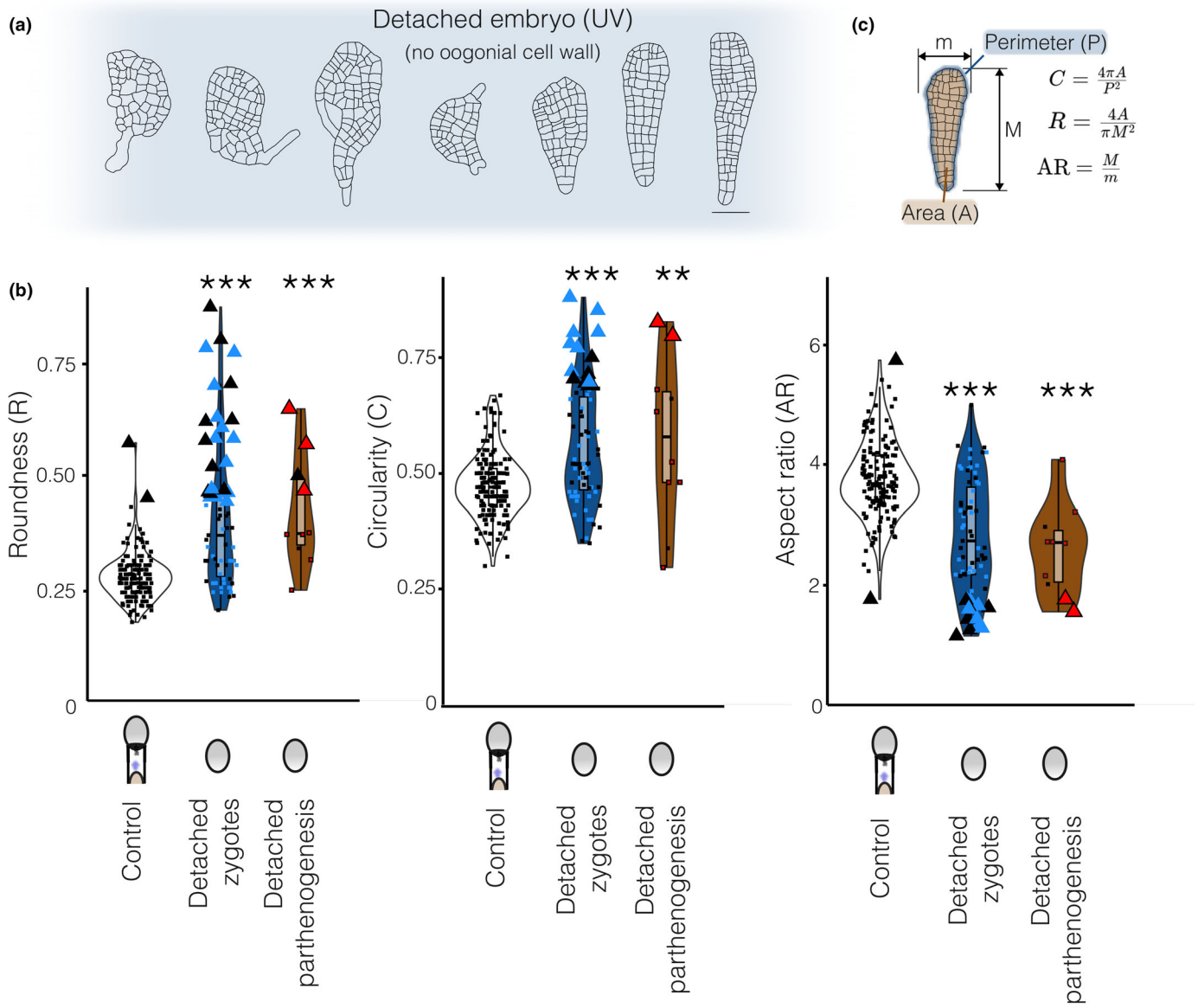

Fig. 4 Detachment from the gametophytic oogonial cell wall affects robustness of the apical–basal direction of the embryo leading to rounder embryos in *Undaria pinnatifida*. (a) Representative detached embryos with aberrant (left) or normal (right) apical–basal patterning. (b) Morphometric parameters for the synchronised control embryos; cut oogonial cell wall; detached zygotes (without oogonial cell wall) and detached parthenogenetic germlings. The violin plots illustrate the distribution of the data for each group, with kernel density estimation showing the data's distribution shape. Embedded within each violin plot is a boxplot that indicates the median and interquartile range with whiskers extending to 1.5 times the interquartile range from the first and third quartiles. Polymerase chain reaction-confirmed crossed embryos are marked in blue and unconfirmed embryos in black, and red symbols denote the presence of the only female marker. Aberrant morphological measurements defined as outliers based on the control distribution (|z-score| > 3) are highlighted with larger triangles. Asterisk denote significant differences with the control (***, $P < 0.001$; **, $P < 0.01$, Supporting Information Table S1). (c) Different morphometric parameters used in this study: AR, aspect ratio; C, circularity; R, roundness.

Attachment to the maternal tissue is required for robust apical–basal patterning of the embryo

In order to test whether attachment of the egg to the maternal oogonium plays a role in early embryogenesis, we detached zygotes from the female gametophyte at the single-celled stage and performed detailed morphometric measurements to follow the early development of the embryo (Fig. 4a,b). To avoid confounding with parthenotes, we identified embryos based on a combination of cell number (30-cell cut-off) and genotyping sex markers.

A range of morphometric parameters were applied (*roundness*, *circularity* and *AR*) to the 4-d-old embryos (Fig. 4c) and the effect of detachment was assessed using GLMMs. Overall, detached embryos were significantly rounder, more circular and had a lower AR compared with control embryos developing in contact with the maternal gametophyte (Fig. 4a,b; Table S1). Because growth is expected to affect the shape of embryos, we included area in the GLM model. Inclusion of the area of embryos in the model did not result in a significantly better fit nor did it affect the significance of the effect of detachment (See Table S1 for GLMMs). Together, these analyses confirmed that detachment explained the difference in morphometric parameters better than the individual size difference between embryos.

The variation of detached phenotypes was larger compared with normal attached phenotypes. Respectively 34%, 20% and 18% of detached embryos qualify as outliers ($|z\text{-score}| > 3$) for roundness, circularity and AR measures compared with the control population (Fig. 4b). Interestingly, these outlier fractions are also present among the few remaining parthenotes that developed > 30 cells at Day 4 (4, 2 and 2 outliers vs control distribution, respectively; $n = 10$; Fig. 4b). This suggests that detachment of both embryos and parthenotes results in a similar ratio of normal and aberrant phenotypes.

Amplification of male and female sex markers in all tested embryos ruled out confounding effects of parthenotes (36, $n = 76$), potentially enriched in the detached fraction. This was further corroborated by the bimodal distribution in cell counts suggesting a clear-cut difference between the slowly developing fraction (suspected parthenotes) and the fast-developing fraction (embryos; Fig. S5). Moreover, growth parameters (number of cells or area) were not affected when putative parthenotes (< 30 cells at Day 4) were omitted from the data (Fig. S4). Because none of the single-celled embryos resulted in successful polymerase chain reaction amplification, we cannot confirm their parthenogenetic nature.

Developmental defects in detached embryos are rescued by the oogonial wall

To discern the cause of the embryo patterning defects, we tested whether the maternal cell wall provides a signal to the developing embryo. To this end, we detached the embryos from live maternal gametophyte cells by cutting the oogonial cell wall. A remnant part of the oogonial cell wall, however, remained attached to the embryo (Fig. 5a,b). When the oogonial cell wall was present, the defect in apical–basal pattern was rescued. Cutting of the oogonial cell wall did not result in significantly different values for morphometric parameters in any of the GLMs (Table S1; Fig. 5b). Only detached embryos without a cell wall and detached parthenotes were morphologically different with significantly higher roundness, circularity and AR (Fig. 4b; Table S1). Our findings are most likely applicable to *Saccharina latissima* because a similar rescue effect of polarity defects could be seen by preserving the oogonial cell wall in a supplementary experiment (Fig. S6), albeit without controlling for potential enrichment in parthenotes.

In the oogamous brown seaweeds *Fucus* and *Dictyota*, the apical–basal polarity is linked to localised cell wall modifications associated with the determination of the basal cell fate. Cell wall modifications can be visualised using TBO, which stains the sulphated F2 fucan fraction at pH 1.0–1.5 (Quatrano & Crayton, 1973; Berger *et al.*, 1994; Bogaert *et al.*, 2017a,b; Fig. 5c). In order to examine whether the apical–basal polarity in *Undaria* is also associated with cell wall modifications, we used TBO on oogonial gametophyte cell wall and the attached embryos. Oogonial cell walls exhibited an intense staining before egg release (Fig. 5c). After release, the oogonial cell wall still retained a more intense blue staining with TBO in comparison with the surrounding vegetative tissue (Fig. 5c).

To test whether TBO staining (at pH 1.5) is conserved we stained other kelps including a member of the Tilpoteridales (Fig. 5d). A similar pattern was observed when staining cultures of Tilopteridales (*Saccorhiza polyschides*) as in *Undaria* (Laminariales) and other Laminariales (*Macrocystis pyrifera* and *Laminaria digitata*) showed similar staining pattern (Fig. 5d,e). Therefore, the apical–basal patterning of the sporophyte of kelps is associated with sulphation of the cell wall of the oogonium (Quatrano & Crayton, 1973), mirroring findings in other brown algal species *Dictyota* (Bogaert *et al.*, 2017b) and *Fucus* (Shaw & Quatrano, 1996; Fig. 6). While *Fucus* and *Dictyota* develop separated from the oogonium and show a deposition of sulphated fucans at the basal pole, kelps show a strong staining in the maternal oogonial cell wall (Fig. 5c–e).

Discussion

Our results show that apical–basal polarity of embryo development in kelp is disturbed when the connection to the maternal gametophyte is disrupted and that preserving only the oogonial cell wall, enriched in sulphated fucans, is sufficient to rescue the defect in robustness of apical–basal patterning.

Maternal guidance of apical–basal patterning is mediated by the oogonial cell wall

Like many complex organisms, kelp embryos develop in close association with the maternal organism. In angiosperms, the embryo is connected to the maternal organism via a suspensor and is influenced by signalling compounds and nutrients (Woudenberg *et al.*, 2024). A role of the maternal tissue in buffering plant embryogenesis against environmental disturbances is suspected but not

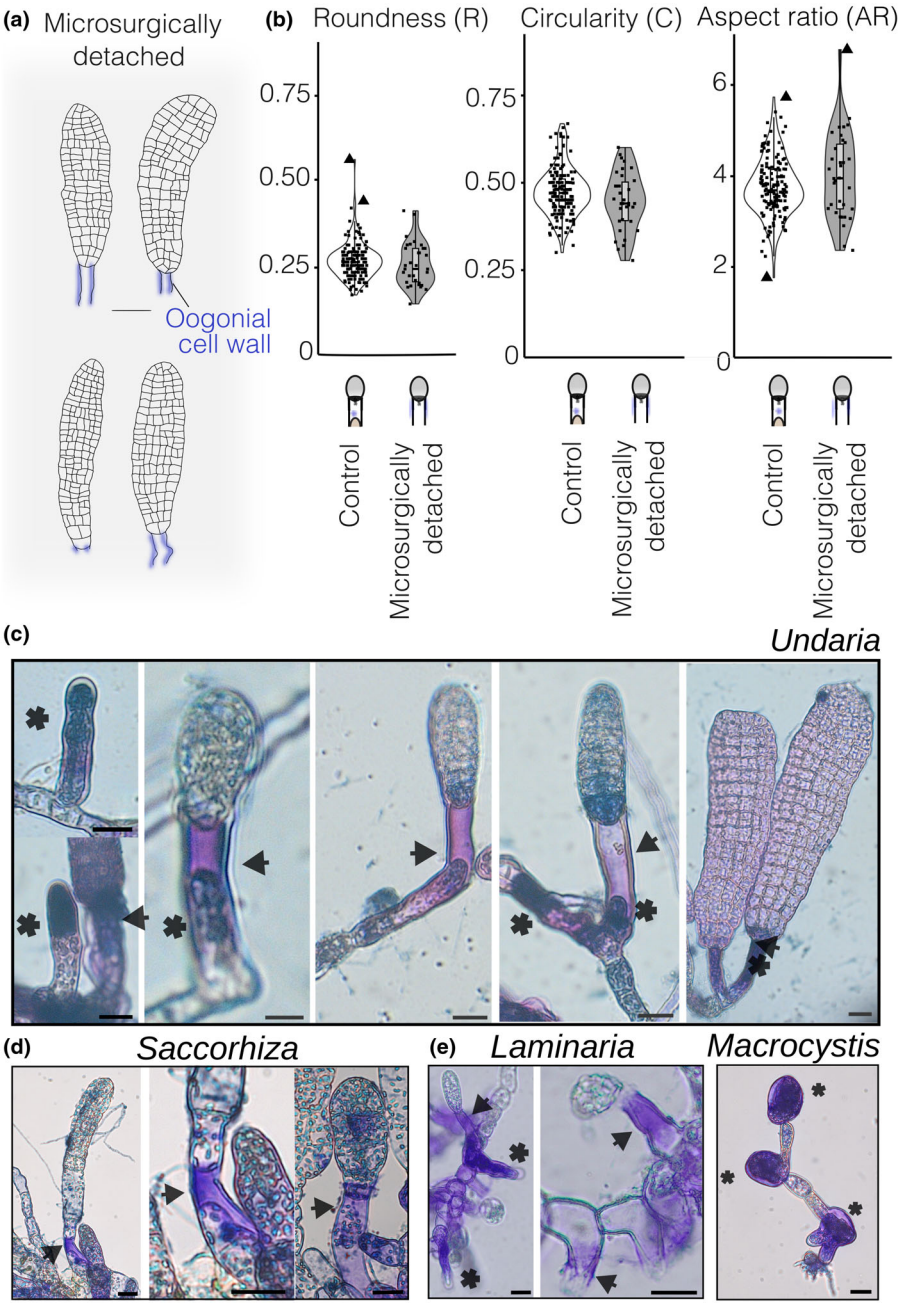

Fig. 5 Preservation of the oogonial cell wall via microsurgical detachments rescues the phenotype in *Undaria pinnatifida* and evolutionary conservation of deposition of Golgi-derived F2 fucans assayed by toluidine blue O (TBO) staining (at pH = 1.5) as a marker for basal cell fate differentiation in the brown *algae*. (a) Representative embryos with oogonial cell wall marked in blue. Bar, 50 μm. (b) Rescue of apical–basal patterning defect if the oogonial cell wall is retained. The violin plots illustrate the distribution of the data for each group, with kernel density estimation showing the data's distribution shape. Embedded within each violin plot is a boxplot that indicates the median and interquartile range. Morphometric parameters did not differ significantly. Aberrant morphological measurements defined as outliers based on the control distribution (|z-score| > 3) are highlighted with larger triangles. (c) TBO in *Undaria pinnatifida* stains the oogonia, oogonial cell wall and sporophytic basal cells. Bars: 20 μm (d, e). Similar staining was observed in other kelp gametophytes such as those of (d) *Saccorhiza polyschides* (Tilopteridales) and (e) *Macrocystis pyrifera* (Laminariales) and *Laminaria digitata* (Laminariales) as in *Undaria pinnatifida*. Asterisks denote staining in oogonia and arrows denote TBO staining in oogonial cell wall bearing the sporophyte. Bars, 20 μm.

fully understood. Different degrees of contact to maternal tissue in nonseed plants are useful to disentangle this interaction (Woudenberg *et al.*, 2024). The kelp maternal association represents a very loose association in which the embryo is not encapsulated as is the case for land plants. Our observations suggest that, nonetheless, the maternal gametophyte provides developmental robustness via the connection to the offspring sporophyte.

By providing one of the first comprehensive descriptions of early embryonic and parthenogenetic kelp development, we are able to compare developmental patterns across different brown algal model organisms. Our observations are in line with the detection of the anchor flagella in *Saccharina* (Motomora, 1991; Klochkova *et al.*, 2019). The cell division pattern of *Undaria* embryos, with cells initially developing a unilayered 2-dimensional blade, are similar as described by Kimura *et al.* (2010), Klochkova *et al.* (2019) and Theodorou & Charrier (2023). Like in *Arabidopsis* (Bayer *et al.*, 2017) and *Dictyota* (Bogaert *et al.*, 2017a,b), the spherical egg undergoes an elongation along the apical–basal axis. Here, we show this elongation is an early process completed for most of the zygotes at the time of detachment and is immediately accompanied by bulge formation. In *Dictyota* and *Fucus* (Fig. 6), cell polarisation of the zygote is only completed late in the cell cycle allowing time to integrate environmental cues. Most likely, the elongation of *Undaria* zygotes depends on the maternal polarity in the polarised egg as very little time is left for environmental signalling.

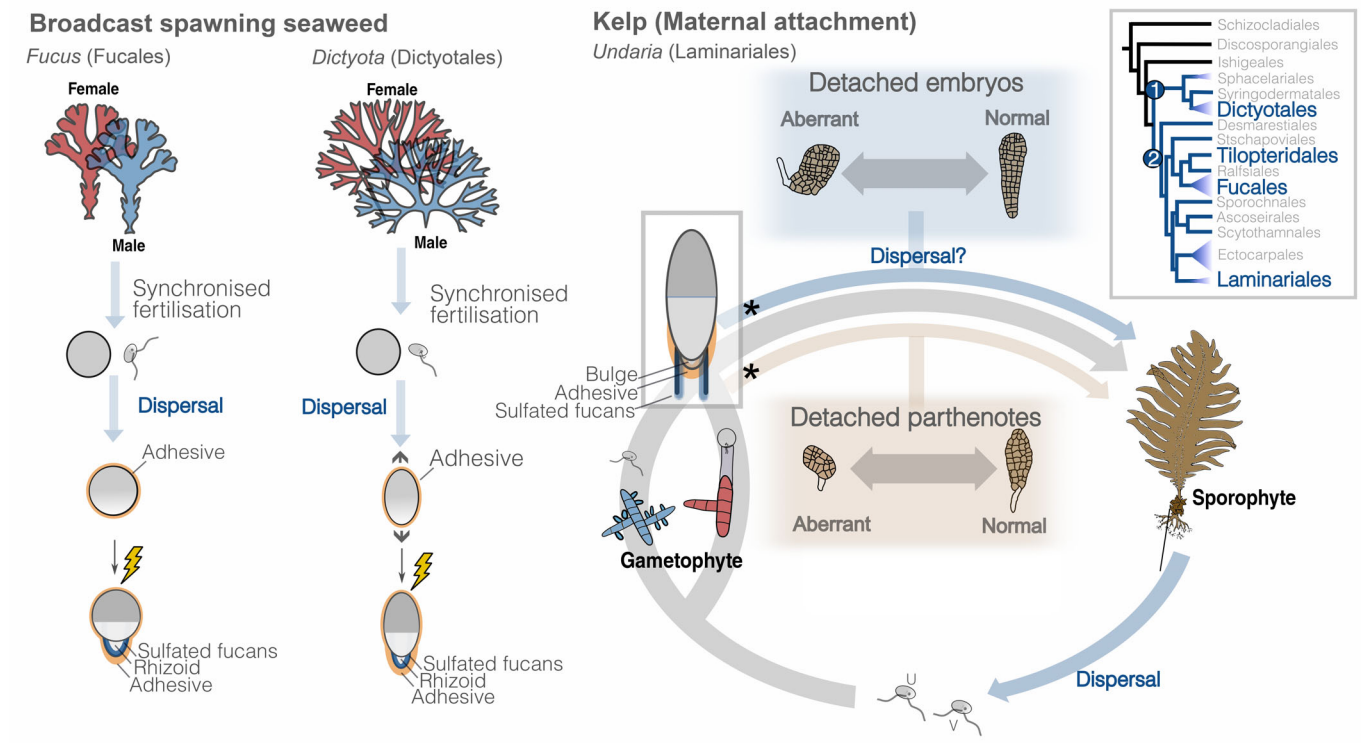

Fig. 6 Development of broadcast spawners *Fucus* and *Dictyota* vs kelp with maternal attachment. (left), *Fucus* and *Dictyota* release gametes synchronously in the surrounding medium producing large zygotes (sporophyte) that will attach using an adhesive and will photopolarise. During asymmetric cell division, the basal cell wall is modified with sulphated fucans. (right) Effects of sporophyte attachment and detachment in kelps. Simplified life cycle of kelps for example *Undaria* focussing on dispersal stages and parthenogenesis. Male gametes are released by male gametophytes (blue) and fertilise female gametes attached to the oogonial cell wall. This oogonial cell wall is modified with sulphated fucans by the female gametophyte (red) before the extrusion of the egg. Spores and dislodged sporophytes are seen as the dispersal stage in kelps, and sporophytes (or parthenotes) develop *in situ* on the gametophyte. The here demonstrated viability of detached seedlings (parthenogenesis and embryo development) might contribute to the dispersal of kelps (blue arrow). Blue and red boxes show variable developmental outcomes of detached embryonic and detached parthenogenetic developmental pathways. Life cycle can be more complex in some species or strains of kelps (Ar Gall *et al*., 1996; Shan & Pang, 2021). Asterisks denote the detachment of embryos or parthenotes. (right, inset) Evolutionary conservation of toluidine blue O (TBO) basal staining in brown algae with the SSDO clade (1) and the BACR clade (2), containing the Laminariales and Tilopteridales.

We show that detaching the zygote from the oogonial cell wall leads to patterning defects, but that a fraction of the detached zygotes is capable of forming well-patterned embryos. This pattern is mirrored in the developmental pattern of parthenotes of *Undaria* and in *Saccharina* (Fig. S6e,f). We conclude that the maternal connection provides developmental robustness to the embryo, but is not essential for its development.

The female gametophyte may make connection with the developing sporophyte. However, direct contact with the live maternal tissue – if restored at all – is only achieved late during embryogenesis and does not occur at all when the neighbouring cells differentiate also in an oogonium. The fact that retaining the oogonial cell wall is enough to rescue the defect in patterning robustness shows that direct interaction by the gametophyte via nutrients or hormones is unlikely to mediate the effect.

We hypothesise that the cell wall of the kelp oogonium is modified in a similar way as described for *Fucus* (Kropf *et al*., 1988; Berger *et al*., 1994; Brownlee & Berger, 1995; Quatrano & Shaw, 1997). In the latter, Golgi-derived vesicles (F-granules) deliver highly sulphated F2 fucans that can be stained using TBO (reviewed in Quatrano & Shaw, 1997). Interestingly, when rhizoid cells were ablated but the basal cell wall was maintained, this cell wall may direct cell fate causing differentiation into the basal cell fate (Berger *et al*., 1994). Here, we showed that, similar to *Dictyota* and *Fucus*, a similar positional correlation between sulphated fucans and basal cell identity occurs in two independently evolving clades of kelps (Laminariales and Tilopteridales), suggesting that this trait is most likely ancestral to the divergence of the two major brown algal clades (SSDO and BACR clade; Figs 5d,e, 6). This further suggests that the mechanism that acts within the developing sporophyte of broadcast spawning algae has been co-opted by the female kelp gametophyte to aid basal cell differentiation in the sporophyte (Fig. 6), therefore acting across life stages in kelps. We cannot exclude, however, that the cell wall may play an (additional) role by keeping the flagellar root in place or alternatively providing a mechanical constricting force enabling bulge formation.

Detachment of eggs in female-only cultures – unexpectedly – induced parthenogenetic development. The addition of gametophyte-conditioned media did not reverse this effect

(Fig. 3c), suggesting that the continuous physical presence of maternal tissue is required to maintain a nonparthenogenetic state. We cannot exclude the possibility that direct contact with maternal cell material in the oogonium plays an inhibitory role similar to the proposed role in apical–basal patterning. However, one may speculate that the disruption of the flagellar connection influences the fate of the maternal centriole pair, which is associated with the flagellar root where it is destined to degrade (Motomura, 1990; Klochkova *et al.*, 2019). Thus, the mechanistic basis for the inhibition of parthenogenesis may involve a role of the maternal centrioles and could be distinct from that causing defects in apical–basal patterning, which should be addressed in future research.

Parthenosporophytes can be discerned from embryos

Parthenotes typically detach more easily (tom Dieck, 1992; Martins *et al.*, 2017; Klochkova *et al.*, 2019) and are rounder (Screiber, 1930; tom Dieck, 1992; Ar Gall *et al.*, 1996; Fang *et al.*, 1979). This aberrant morphology and tendency to detach are used as indications for parthenogenesis (tom Dieck, 1992; Martins *et al.*, 2017; Liboureau *et al.*, 2024). While most parthenotes are not viable, a proportion of them may grow into reproductive adult seaweeds (Fang *et al.*, 1979; Lewis *et al.*, 1993; Müller *et al.*, 2019; Camus *et al.*, 2021; Shan *et al.*, 2021). These observations often co-occur with polyploidy, suggesting that similarly to *Ectocarpus* (Bothwell *et al.*, 2010) endoreduplication may occur during parthenogenesis (Lewis *et al.*, 1993; Shan *et al.*, 2021). While the (largest) individuals we obtained in culture flasks were all haploid, we cannot exclude that diploidisation occurs in other parthenotes.

Being correlated to both the treatment (detachment) and the outcome (abnormal development), parthenogenesis has to be controlled for as a potential confounding variable. The issue of parthenotes is stressed by the observation that upon detachment a 20-fold increase in parthenogenetic development occurs in unfertilised eggs from female-only cultures (Fig. 3c). Here, we confirmed that in *Undaria* the detached fractions were not enriched in parthenotes. While initial experiments were conducted in *Saccharina* (Fig. S6), an enrichment in parthenotes was suggested by the slower growth of the detached fraction. Therefore, we resorted to an *Undaria* strain which shows much lower parthenogenesis rates.

The kelp (partheno-)sporophyte as a dispersal stage

Understanding the mechanisms of kelp dispersal is important to (1) estimate the degree of gene flow from commercially grown kelps to native populations, (2) understand the ways of dispersal in kelp restoration projects and (3) understand the dispersal vectors for non-native kelps such as *U. pinnatifida* (Hay, 1990). Kelps are thought to disperse via the zoospore stage (short-range) and via dislodged fertile sporophytes (long-range; Forrest *et al.*, 2000; Vanderklift *et al.*, 2020; Edwards, 2022). Due to the physical connection to the maternal gametophyte, the zygote and embryo are not considered as dispersal vectors. The observation that embryos may lead to individuals bearing rhizoids (Fig. 4a), despite significant patterning defects, raises the question to what extent detached development is relevant in the field and contributes to kelp dispersal in a similar fashion as in broadcast spawners (Fig. 6).

Additionally, detachment of unfertilised eggs from the gametophyte triggers parthenogenetic development. Because at least some parthenotes of kelp species, including *U. pinnatifida*, have been shown to develop normally and even to become fertile (Fang *et al.*, 1979; Lewis *et al.*, 1993; Müller *et al.*, 2019; Camus *et al.*, 2021; Shan *et al.*, 2021), detachment of parthenotes by for example wave action or swaying seaweeds may provide an additional dispersal stage. Reproductive parthenogenetic kelp sporophytes have been observed in natural populations (Oppliger *et al.*, 2007; Klochkova *et al.*, 2017). However, while fully parthenogenetic range-edge populations are described for other brown algae (Hoshino & Kogame, 2019; Hoshino *et al.*, 2021), population genetic studies of *Undaria* have as yet not reported widespread clonality (Shan & Pang, 2018; Guzinski *et al.*, 2018, Shan *et al.*, 2022), indicating that the role of parthenogenesis in these populations is not a dominant factor.

Bringing the kelp model up to par with broadcast spawning seaweeds

In broadcast spawners, it is easy to obtain large populations of synchronously developing zygotes free from viable parthenotes and maternal tissue, making them suitable models for studying the installation of apical–basal polarity and the asymmetric cell division (Coelho & Cock, 2020; Bogaert *et al.*, 2023). The attachment of eggs to the maternal gametophyte and the repeated gamete release over days obscure different cohorts (Forbord *et al.*, 2018; Theodorou *et al.*, 2021). First, we mitigated the negative implications of attachment on fertilisation and polarisation assays. Our approach allowed us to synchronise fertilisation. Second, we examined detached embryogenesis compared with normal (attached) embryogenesis. Detached development might still be considered for some applications such as automated phenotyping, as long as the polarity defects are taken into account or irrelevant to the assay (e.g. individual photosynthetic rates; compound production). Third, we demonstrate how to address the issue of parthenogenesis using the growth delay and sex markers.

In conclusion, we showed that apical–basal polarity in the kelp embryo is less robust when detached, resulting in defects in apical–basal patterning. We show that the robustness of apical–basal polarity is restored when the cell wall remains attached, suggesting a role for the connection with the cell wall of the oogonium. We further assessed the hypothesis that the robust apical–basal patterning is influenced by the deposition of Golgi-derived cell wall material by TBO staining, a method previously used to demonstrate the establishment of basal cell fate in brown algae as in the case for *Fucus* (Shaw & Quatrano, 1996; Quatrano & Shaw, 1997). The here proposed working hypothesis suggests that the fate of the basal cell is determined by substances secreted into the oogonial cell wall by the maternal gametophyte reminiscent to the mechanism in the brown alga *Fucus* (Kropf *et al.*, 1988; Berger *et al.*, 1994) and that these positional cues contribute to the robustness of developmental patterning.

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Competing interests

None declared.

Author contributions

ED contributed to the investigation, methodology, visualisation, writing – original draft, and writing – review and editing. YM, FMG, JFMB and EEZ contributed to the investigation, methodology, and writing – review and editing. DL contributed to the investigation, visualisation and writing – review and editing. TB: conceptualisation and writing – review and editing. SMC and ODC: conceptualisation, funding acquisition, supervision, methodology and writing – review and editing. KAB: conceptualisation, investigation, supervision, methodology, visualisation, writing – original draft and writing – review and editing. SMC, ODC and KAB contributed equally to this work.

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Data availability

Data are available on figshare: doi: 10.6084/m9.figshare.26009950.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Representative developing zygote undergoing elongation along the apical–basal polarity vector and asymmetric cell division.

Fig. S2 Representative developing zygote undergoing elongation along the apical–basal polarity vector.

Fig. S3 Sporophyte develops during first 3–4 d only in direct contact with the oogonial cell wall of the female.

Fig. S4 Results of flow cytometry analysis to estimate DNA content of *Undaria pinnatifida*.

Fig. S5 Slow cell division as a marker for parthenogenesis vs normal development (detached or attached).

Fig. S6 Rescue effect of the cell wall can also be observed in *Saccharina latissima*.

Table S1 Table describing results from GLM modelling of respectively circularity, roundness and AR in response to growth and detachment.

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