

1 **Emergence of the subapical domain is associated with the midblastula** 2 **transition**

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8 **Key words:** cortical domains, epithelial domains, subapical, Canoe, midblastula
9 transition, zygotic genome activation

10 **Abstract**

11 Epithelial domains and cell polarity are determined by polarity proteins which are
12 associated with the cell cortex in a spatially restricted pattern. Early *Drosophila*
13 embryos are characterized by a stereotypic dynamic and de novo formation of cortical
14 domains. For example, the subapical domain emerges at the transition from syncytial
15 to cellular development during the first few minutes of interphase 14. The dynamics
16 in cortical patterning is revealed by the subapical markers Canoe/Afadin and ELMO-
17 Sponge, which widely distributed in interphase 13 but subapically restricted in
18 interphase 14. The factors and mechanism determining the timing for the emergence
19 of the subapical domain have been unknown. In this study, we show, that the restricted
20 localization of subapical markers depends on the onset of zygotic gene expression. In
21 contrast to cell cycle remodeling, the emergence of the subapical domain does not
22 depend on the nucleo-cytoplasmic ratio. Thus, we define cortical dynamics and

specifically the emergence of the subapical domain as a feature of the midblastula transition.

Author summary

Midblastula transition is a paradigm of a developmental transition. Multiple processes such as cell cycle, cell mobility, onset of zygotic gene expression, degradation of maternal RNA and chromatin structure are coordinated to lead to defined changes in visible morphology. The midblastula transition in *Drosophila* embryos is associated with a change from fast nuclear cycles to a cell cycle mode with gap phase and slow replication, a strong increase in zygotic transcription and cellularization. The timing of the processes associated with the midblastula transition are controlled by the onset of zygotic gene expression or the nucleocytoplasmic ratio. Here we define the patterning of cortical domains, i. e. the emergence of a subapical domain as a novel feature of the midblastula transition whose appearance is controlled by the onset of zygotic transcription but not the nucleocytoplasmic ratio. Our findings will help to gain further understanding of the coordination of complex developmental processes during the midblastula transition.

Introduction

The cell cortex underlies the plasma membrane and consists of a layer of F-actin and associated proteins, including actin nucleators, regulators and myosin motors. Proteins, such as ERM proteins, link F-actin to the plasma membrane (1). Typical for epithelial cells are cortical domains, which contain marker proteins specific for the respective domain in addition to the general set of cortical proteins. For example, Par-3/Bazooka (Baz) typically marks the subapical domain, whereas Par-1 marks the lateral domain (2,3). Although mutual exclusion of such marker proteins has been shown to maintain boundaries between two domains in some cells, the mechanism for

48 initial establishment of the domains and pattern formation is not well defined. The de
49 novo appearance of the first epithelium during cellularization in *Drosophila* embryos,
50 provides an excellent model to study the initial formation of cortical domains and
51 epithelial polarization (4).

52 Following a syncytial phase of development with rapid nuclear cycles typical for
53 insects, the first epithelium forms after about two hours of embryonic development as
54 a morphologically obvious feature marking the transitions from syncytial to cellular
55 blastoderm (5–7). This morphological change, often referred to as midblastula
56 transition (MBT) is associated with several cellular processes that appear to be
57 coordinated, including remodeling of the cell cycle, transition to a slow mode of DNA
58 replication, heterochromatin formation, ingression of the cellularization furrow,
59 elongation of the nuclei, and importantly activation of the zygotic genome (6,8,9).
60 Concerning epithelial polarization it is important to note that the number of cortical
61 domains increases during the transition from two cortical domains (caps and intercaps)
62 in interphase 13 (10,11) and three domains (apical, lateral, basal) during mitosis (12)
63 to the typical four domains. A dedicated subapical region positioned between the
64 apical and lateral domains emerges for the first time in development in interphase 14
65 (3,8).

66 It is unknown, if and how the emergence of the subapical domains is linked or
67 coordinated with the other processes associated with the midblastula transition. It has
68 been previously shown that zygotic transcription initiates the cell cycle remodeling
69 and is required for cellularization (13). The changes are due to specific zygotic genes,
70 e. g. *slam*, *nullo*, *frs* or to global signals such as transcription associated DNA
71 replication stress and DNA checkpoint activation (13). The emergence of the subapical
72 domain has not been investigated in this context, so far.

73 The earliest marker proteins for the prospective subapical domain during onset of
 74 cellularization are Canoe (Cno, Afadin in vertebrates) and the unconventional GEF
 75 complex ELMO- Sponge (8,14), which act upstream of Canoe possibly via control of
 76 the small GTPase Rap1. Both Canoe and ELMO-Sponge are widely distributed during
 77 the syncytial interphases and mitoses (nuclear cycles 10–13). Canoe is detected in cap
 78 and intercap regions, whereas the ELMO-Sponge complex marks the actin caps and
 79 control their formation (8). This disc-like pattern in pre-MBT interphases changes to
 80 a ring-like pattern in interphase 14, when ELMO-Sponge initiate restriction of Canoe
 81 to the prospective subapical region. Only during the course of cellularization, the
 82 typical subapical proteins Bazooka/Par-3 and Armadillo (Arm, β -Catenin in
 83 vertebrates) are enriched in the subapical region (15–17).

84 In this study, we investigate the role of zygotic gene expression and cell cycle
 85 remodeling for the formation of the subapical domain. As Bazooka feeds back on
 86 subapical restriction of Canoe later in cellularization, we tested the function of this
 87 genetic interaction for the initial emergence of the subapical region. We show that the
 88 localization of early subapical domain markers like ELMO-Sponge and Canoe
 89 depends on onset of zygotic expression but not cell cycle remodeling and not on
 90 *bazooka* during early cellularization.

91 **Results**

92 **Change of Canoe distribution pattern at the onset of interphase 14**

93 The subapical cortical domain emerges during the transition from syncytial to cellular
 94 blastoderm for the first time during embryonic development. During this process the
 95 localization pattern of the actin binding protein Canoe changes from a dispersed
 96 pattern at the actin caps to a coalesced pattern at the prospective subapical domain
 97 within about five minutes of the onset of cellularization in interphase 14 (Figure 1A)

(8). Subapical restriction of Canoe depends on the small GTPase Rap1 and the unconventional guanyl nucleotide complex ELMO-Sponge, which undergoes a relocation from discs in interphase 13 to rings in interphase 14 (8). New cellularization furrows form between the daughter nuclei. After reached longest extension in metaphase, these furrows gradually retract in the second half of mitosis to a length of about 3 μm (32,33) (Figure 1B). We applied our live imaging assay with embryos expressing the subapical marker CanoeYFP and basal marker CherrySlam to reveal the kinetics of marker segregation. Axial stacks were recorded and computationally projected to sagittal sections. During mitosis, Cherry Slam was detected at the tip of the metaphase furrow, whereas CanoeYFP was spread along the full length (Figure 1C). It is important to note the difference between “old” cellularization furrows, which arise from retracting metaphase furrows, and “new” cellularization furrows, which ingress between daughter nuclei. In “new” cellularization furrows CanoeYFP associates within minutes to the in folding membrane. In contrast, Canoe distribution is becomes subapically restricted at “old” furrows starting from a wide distribution along the furrow (Figure 1C). CherrySlam remains at the tip of “old” furrows, and gradually appears at the tip of “new” furrows (Figure 1C) (8). Although we and others have uncovered the mechanism for subapical restriction of Canoe (3,8,15,17,34), the factors determining the timing have not be studied.

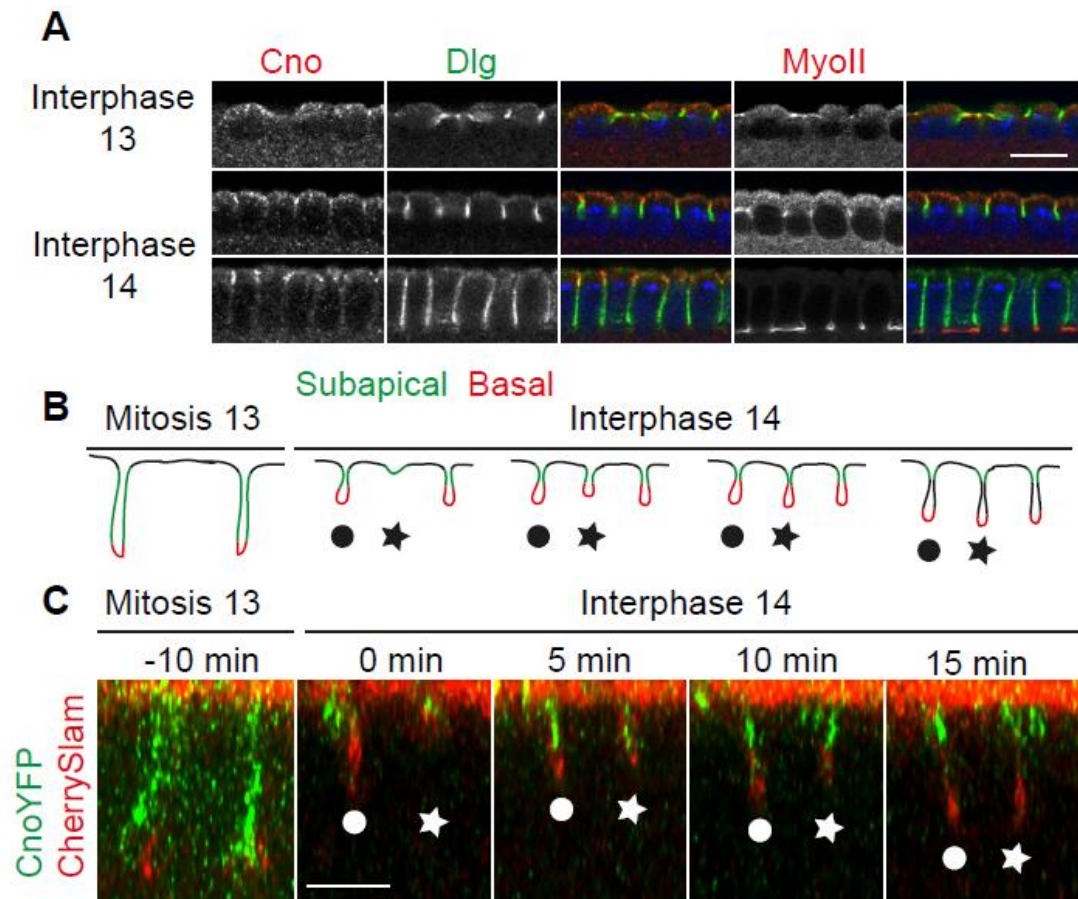


Figure 1 Canoe relocalizes in old cellularization furrows arising from retracting metaphase furrows. (A) Fixed wild type embryos during interphases 13 and 14 as indicated stained against Canoe (grey/red), Dlg (grey/ green), MyosinII (grey/ red) and DNA (blue). (B) Scheme of metaphase and cellularization furrows during mitosis 13 and switch to interphase 14 as indicated. Proteins localizing to the subapical domain during interphase 14 (green) localize to the whole cortex of metaphase furrows. After mitosis 13 metaphase furrows retract and come to a halt forming “old furrows” (circle) while “new furrows” form between (star). The furrows move inwards synchronously when they have reached the same length. (C) Living embryos expressing CanoeYFP (green) and CherrySlam (red) to mark subapical and basal domains. Orthogonal views are shown. Stages are as indicated. Scale bar 10 μ m.

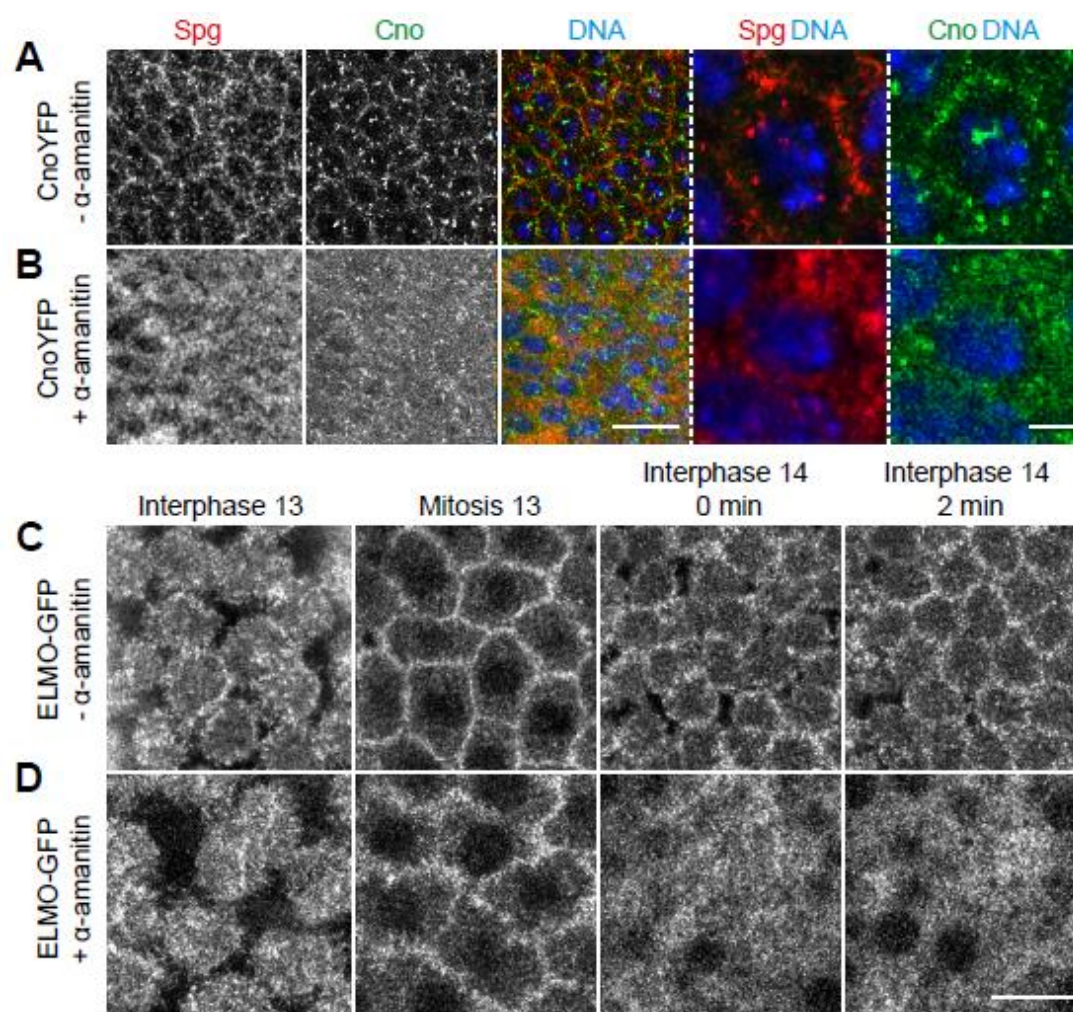
118 Formation of the subapical domain depends on zygotic gene expression but not 119 the nucleo-cytoplasmic ratio

120 The change from syncytial to cellular blastoderm at the onset of cell cycle 14 and
121 cellularization requires zygotic gene expression (35). Although it is clear that
122 cellularization depends on zygotic gene expression, its functional relationship to the
123 emergence of the subapical domain has not been investigated. ELMO, Sponge, Rap1
124 and Canoe are maternally derived proteins, whose total levels are assumed not to
125 change much. Rather, their distribution on the plasma membrane is controlled by post-
126 translational mechanisms.

127 We first asked whether the spatial restriction of subapical markers depended on zygotic
128 transcription. We analyzed embryos, in which zygotic transcription was blocked by α -
129 amanitin, an efficient inhibitor of RNA polymerase II. Injection of α -amanitin impairs
130 furrow invagination and cellularization (35). Early embryos expressing CanoeYFP
131 were injected with α -amanitin prior to cellularization (Figure 2A). In fixed control
132 embryos, Sponge and CanoeYFP marked the invaginating furrows in a hexagonal
133 pattern, enclosing the nuclei as visible in surface views (Figure 2A). In contrast, no
134 spatial restriction of Sponge and CanoeYFP was detected in injected embryos in
135 interphase 14, indicating that the restriction of the subapical markers depends on
136 zygotic transcription (Figure 2B). To better resolve the dynamics and staging of the
137 embryos, we recorded time lapse images of embryos expressing ELMO-GFP (Figure
138 2C-D). Control embryos showed a stereotypic ELMO localization at caps during
139 syncytial blastoderm stages and transition to subapical rings during the first few
140 minutes of cellularization in interphase 14 (Figure 2C). In embryos treated with α -
141 amanitin, the cap staining during syncytial blastoderm stage was comparable to control
142 embryos. In contrast, the ELMO-GFP signal remained widely distributed over the
143 whole cortex without any obvious spatial restriction after mitosis 13 (Figure 2D). This

144 loss of restriction of ELMO-GFP was observed at a time when the morphologically
145 visible furrows has not yet formed in control embryos. Our data indicate that spatial
146 restriction of ELMO, Sponge and Canoe in interphase 14 and thus formation of the
147 subapical domain depends on zygotic transcription.

148



149

150 Figure 2 Zygotic gene expression is necessary for the formation of the subapical domain during
151 cellularization. (A, B) Fixed non-injected (A) and α -amanitin-injected (B) embryos expressing
152 CanoeYFP stained against Sponge (grey/ red), CanoeYFP (grey/ green) and DNA (grey/ blue)
153 during interphase 14. Merged images and zoom-ins are shown in right panels. (B, D) Top
154 views of images from time lapse movies of non-injected (C) and α -amanitin-injected (D)

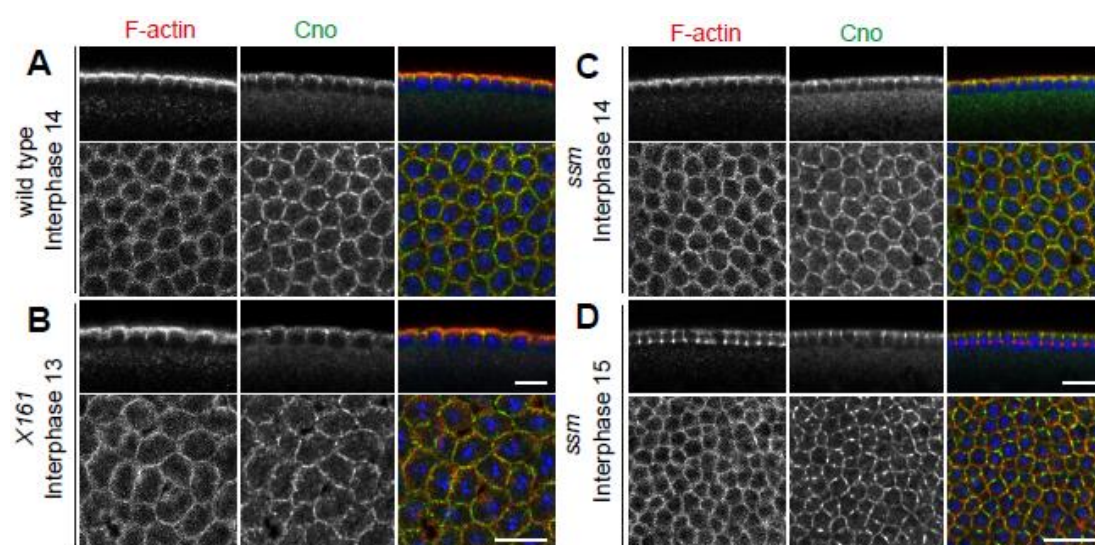
embryos expressing ELMO-GFP. Time points are indicated above the images. Scale bars 10 μm .

A second obvious timing mechanism beside onset of zygotic transcription during the transition from syncytial to cellular blastoderm is the nucleocytoplasmic ratio. Haploid embryos undergo an extra nuclear division and cellularize only in interphase 15 (36) (Figure 3C, D). It was previously reported that haploid embryos showed features of a cellularization furrow already in interphase 14, i. e. transient basal accumulation of Myo II at the furrow tip (37,38). Cortical domains have otherwise not been specifically investigated in haploid embryos, yet. We fixed and stained haploid embryos from *sésame* (*ssm*, *Hira*) females (21) for Canoe and F-actin. We detected specific subapical restriction of Canoe in cellularizing embryos in interphase 14 as well as in interphase 15 (Figure 3 C, D). Consistent with the previously reported basal restriction of MyoII, these data suggest that the transient furrow during interphase 14 in haploid embryos contains a patterned cortex with a subapical region. We conclude that the emergence of the subapical and basal domains does not depend on the nucleocytoplasmic ratio.

A third timer associated with the midblastula transition is the remodeling of the fast nuclear cycle to a slow cell cycle, which depends on the onset of zygotic transcription (7). We tested whether subapical Canoe restriction would respond to a precocious zygotic transcription and precocious cell cycle remodeling. We analyzed embryos from *RPII215^{X161}* germline clones, which precociously start zygotic transcription, cellularize already in interphase 13, and further develop with half of the number of nuclei (9). By staining of fixed embryos, we detected a normal pattern of F-actin and subapical restriction of Canoe in embryos cellularizing in interphase 13 (Figure 3A, B).

179 In summary, our data indicate that the formation of the subapical domain is a regulated
180 feature of the midblastula transition, which responds to zygotic gene expression but
181 not on the nucleocytoplasmic ratio. The failed spatial restriction in embryos with
182 impaired zygotic transcription may be due to the absence of one or multiple specific
183 zygotic factors, which control the distribution pattern of ELMO- Sponge complex, for
184 example. Alternatively, failed spatial restriction may be a consequence of zygotic
185 transcription, such as high polymerase activity or transcription dependent DNA
186 replication stress. Although our time lapse analysis of ELMO-GFP and CanoeYFP
187 indicates that subapical restriction precedes furrow ingression, we do not exclude the
188 possibility that subapical restriction is a consequence of furrow formation due to the
189 limited resolution of our assay.

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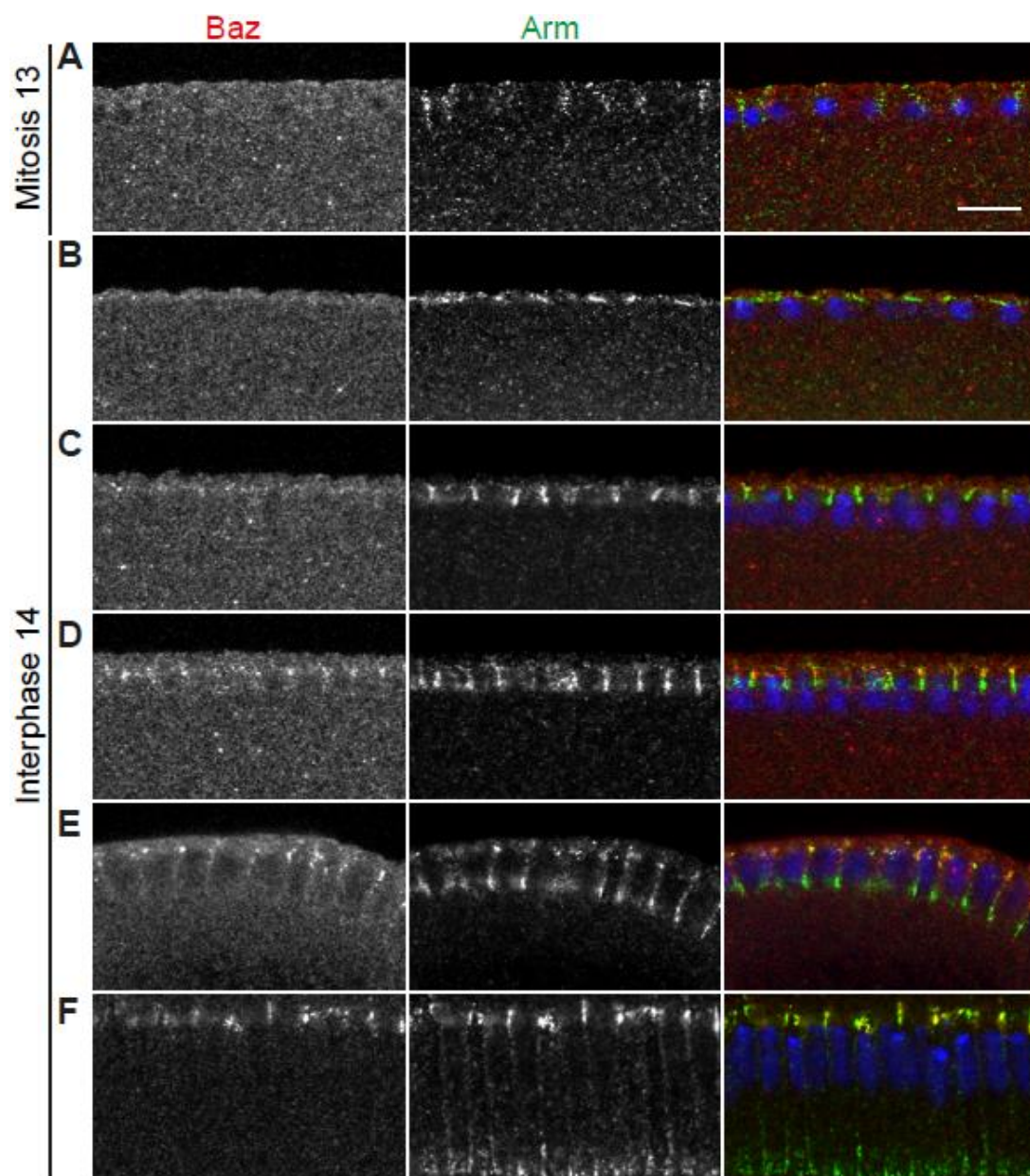


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192 Figure 3 Cortical domain formation depends on zygotic gene expression and not on
193 nucleocytoplasmic ratio. (A-D) Fixed wild type (A), *X161* (B) and *sésame* (C, D) embryos
194 stained against F-actin (grey/ red), Canoe (grey/ green) and DNA (blue). Merged images are
195 shown in right panels, sagittal sections in top panels and accompanying top views in lower
196 panels. Stages are as indicated. Scale bars 10 μ m.

197 **Bazooka does not regulate subapical Canoe localization during early** 198 **cellularization**

199 Bazooka is a potential zygotic factor controlling subapical restriction of ELMO-
200 Sponge and Canoe, since Bazooka has a maternal and zygotic expression. Previous
201 work revealed a positive feedback mechanism during late cellularization in which
202 subapical restriction of Canoe becomes partially dependent on *bazooka* (15). We asked
203 whether this feedback interaction was active also during the onset of cellularization.
204 Firstly, we analyzed the distribution of Bazooka and Armadillo which marks E-
205 Cadherin junctions in fixed wild type embryos. For this overview, we imaged all
206 embryos with the same laser settings to compare protein localization and amounts in
207 different stages. With these settings, we did not detect Bazooka at Armadillo positive
208 metaphase furrows during mitosis 13 (Figure 4A). The subapical restriction of
209 Armadillo matures during the course of cellularization starting from an initially wide
210 distribution along the furrow. The basal junction, in comparison, was detected very
211 early on as reported previously (3,39)(Figure 4B–D). A clear subapical Bazooka
212 restriction was first detected during cellularization when the furrows extended to
213 around half the length of the elongated nuclei (Figure 4D). Remarkably, at this time
214 point subapical Armadillo enrichment was not visible yet. During the course of
215 cellularization Bazooka puncta persisted at the subapical position colocalizing with
216 Armadillo (3,40) (Figure 4E, F). The lack of a subapical Bazooka signal during early
217 cellularization does not support an early function of the feedback regulation on Canoe.



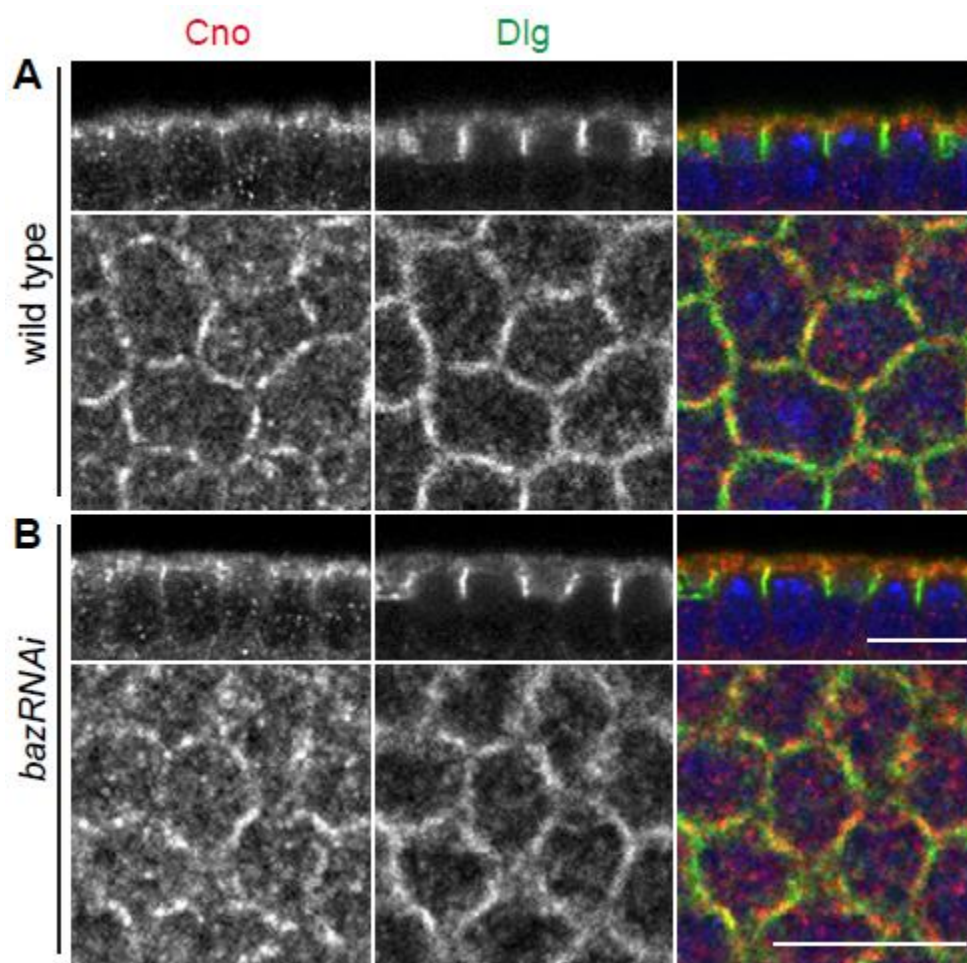
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219 Figure 4 Subapical enrichment of Bazooka and Armadillo during cellularization. (A-F) Fixed
 220 wild type embryos during mitosis 13 (A) and interphase 14 (B-F, from early to late
 221 cellularization). Embryos were stained in the same tube against Bazooka (grey/red), Armadillo
 222 (grey/ green) and DNA (blue) and imaged with same laser settings to estimate different protein
 223 amounts in different stages. Scale bar 10 μ m.

224 To clarify the relation of Canoe and Bazooka in functional terms, we depleted *bazooka*
 225 by RNAi and analyzed fixed and stained embryos (Figure 5). RNAi depletion is
 226 functional as indicated by the loss of Bazooka staining and the later phenotype with

227 holes in the amnioserosa (Supplemental figure 1). Subapical Canoe enrichment was
 228 comparable in wild type controls and *bazookaRNAi* embryos during early
 229 cellularization whereas Canoe localization was affected as described before in early
 230 gastrulating embryos (15) (Supplemental figure 1B). Based on these data we conclude
 231 that the Bazooka-Canoe feedback loop becomes activated only during the course of
 232 cellularization and is not involved in the initial subapical restriction of Canoe.

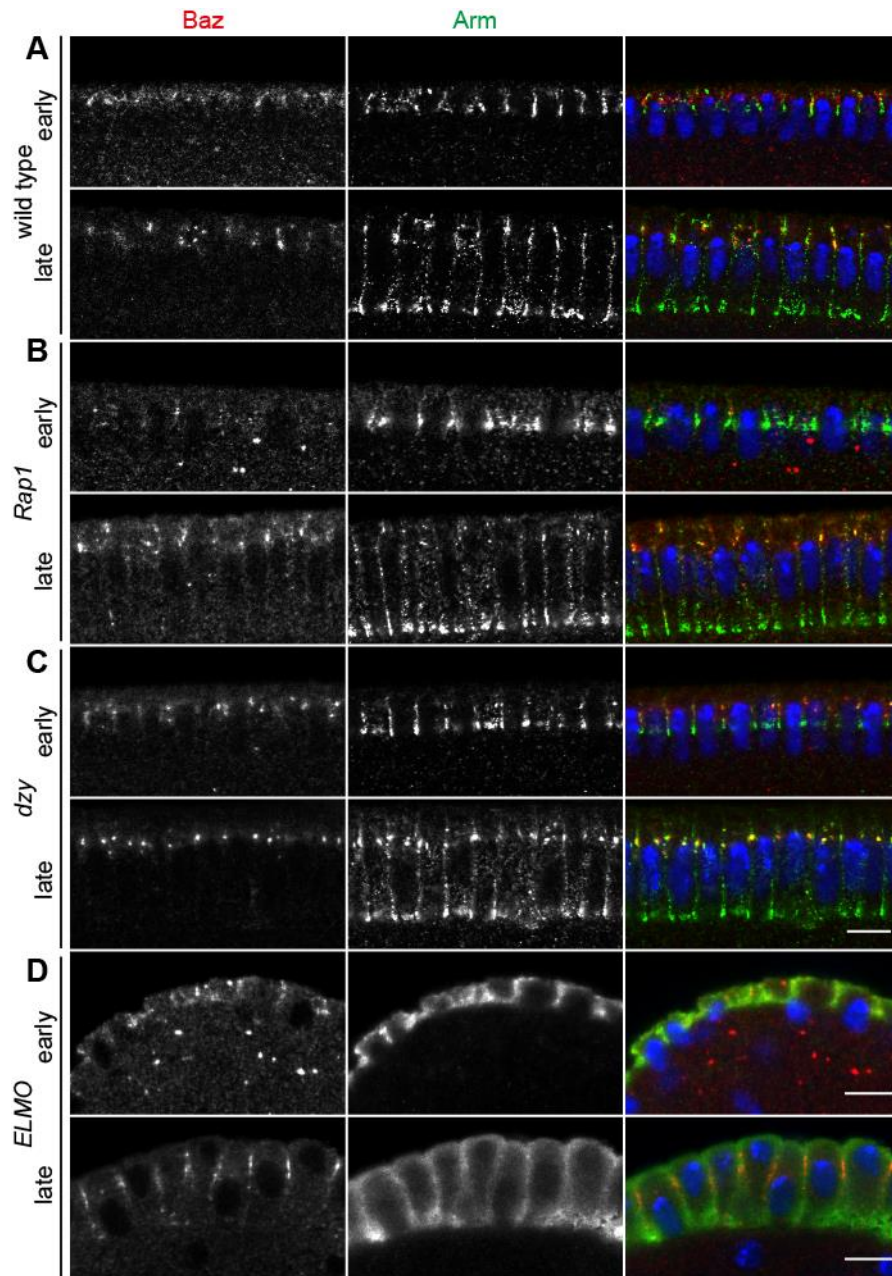
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 235 Figure 5 Canoe localization is not affected by Bazooka during early cellularization. (A-B)
 236 Fixed wild type (A) and *bazRNAi* (B) embryos during early cellularization stained against
 237 Canoe (grey/ red), Dlg (grey/ green) and DNA (blue). Side views are shown in upper panels
 238 and corresponding top views in lower panels. Scale bars 10 μ m.

239 **Subapical Bazooka enrichment is controlled by the unconventional GEF ELMO-** 240 **Sponge**

241 Initial subapical restriction of Canoe is controlled by the unconventional GEF complex
242 ELMO-Sponge and the GTPase Rap1. We asked whether this functional dependence
243 also holds true for Bazooka and Armadillo. By analysis of fixed embryos, we found
244 that both *Rap1* and *ELMO* were required for subapical restriction of both Bazooka and
245 Armadillo. Bazooka and Armadillo staining was dispersed along the lateral furrow in
246 embryos from females with ELMO as well as Rap1 germline clones consistent with
247 previous reports (15) (Figure 6B, D). Conversely Bazooka and Armadillo did not
248 depend on a different Rap1GEF, *dizzy*, (Figure 6C) consistent with our previous report
249 that subapical restriction of Canoe did not depend on *dizzy* (8). These findings confirm
250 the earlier described pathway of Bazooka being downstream of the unconventional
251 Rap1 GEF complex ELMO-Sponge, Rap1GTPase and Canoe during early and late
252 cellularization.



253

254 Figure 6 Subapical enrichment of Baz and Arm is perturbed in *Rap1* and *ELMO* but not in *dzy*
255 mutants. (A–D) Fixed cellularizing wild type (A), *Rap1* (B), *dzy* (C) and *ELMO* (D) embryos
256 stained against Baz (grey/ red), Arm (grey/ green). DNA is shown in blue. (A) Wild type
257 embryos during early and late cellularization showed subapical Baz and Arm enrichment. (B)
258 Baz puncta are spread along the lateral membrane in early and late cellularization of *Rap1*
259 embryos. The subapical Arm enrichment was lost whereas basolateral enrichment was still
260 visible. (C) The subapical enrichment of Baz and Arm is not perturbed in early and late

cellularizing *dzy* mutant embryos. (D) Baz and Arm subapical localization is lost in *ELMO* mutants during early and late cellularization. Scale bars 10 μ m.

Discussion

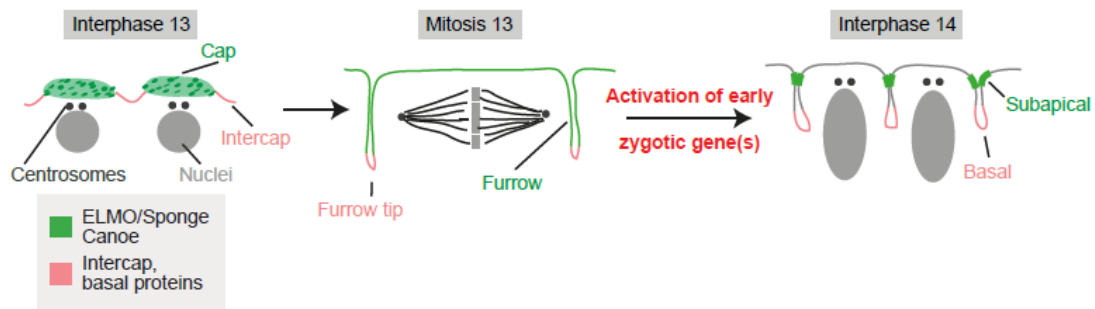
In this study we focused on the function of zygotic gene expression on the formation of the subapical domain during onset of cellularization. Next to the already known fact, that *ELMO*-Sponge and Canoe localize to newly forming cellularization furrows at onset of interphase 14 (8), we were able to show, that Canoe quickly changes its distribution pattern at “old” cellularization furrows (Figure 7). Although we were able to show Canoe preceding Bazooka to localize to the newly forming furrow and subapical domain, with Bazooka being gradually enriched at the subapical domain during the course of cellularization, we confirmed that Canoe is initially restricted independently of Bazooka. In this analysis we relayed on RNAi mediated depletion, since *bazooka* has an essential early function in germline determination (41–43).

The features of the midblastula transition include deceleration and remodeling of the cell cycle, degradation of maternal products and the switch from syncytial to a cellular blastoderm and the onset of zygotic gene expression (13,36,44,45). As new feature of the morphological changes associated with the midblastula transition we describe here a change in cortical patterning, i. e. the emergence of the subapical domain. Although the restriction of subapical markers precedes formation of a morphologically visible furrow, the apposition of two plasma membranes in initial furrow formation could be the cause of marker restriction, given the limited morphological resolution of our assays. A hint could come from the “old” cellularization furrows that arise from metaphase furrows, which were still detectable in α -amanitin injected embryos by *ELMO*-GFP. Even at the positions of the old furrow the spatial restriction is lost. A limitation to this argument is again the limited insight into the cellular morphology

286 and dynamics, as the dynamics of the metaphase furrow in embryos lacking zygotic
287 transcription is not clear. A more defined insight into the timing by zygotic gene
288 expression comes from our investigations of embryos with precocious onset of zygotic
289 gene expression. We could detect Canoe at forming cellularization furrows whenever
290 zygotic gene expression was initiated.

291 The next arising question is which zygotic gene or genes could be responsible for
292 relocalization of the subapical domain proteins with onset of cellularization. Among
293 the described early zygotic genes like *slam*, *nullo*, *bottleneck* and *serendipity-α* no
294 such phenotypes have been described yet (8,18,46–48). However, as general
295 morphology was the primary assay for the screen of zygotic genes (49,50), the
296 subapical determinant might have been missed. A molecular screen of aneuploid
297 embryos for mislocalization of subapical domain proteins may allow the identification
298 of these genes, for example. Although *bazooka* is already maternally expressed, it
299 seems to take over the function as the subapical determinant only later during in
300 cellularization (15,16). Although, it is not clear how much the expression levels were
301 reduced in *bazookaRNAi* embryos, we were not able to detect Bazooka protein by
302 staining.

303 Taken together, we were able to show, that the formation of the newly established
304 subapical domain is a novel feature of the midblastula transition, which depends on
305 the onset of zygotic transcription. We propose the hypothesis, that a yet unknown
306 zygotic gene triggers the signaling cascade for subapical domain formation involving
307 ELMO-Sponge, Rap1 and Canoe.



308

309 Figure 7 Model of cortical domain protein dynamics from interphase 13 to interphase 14.

310 During interphase 13 cortical domain proteins divide in cap (green) and intercap (red)

311 localization, later subapical proteins like Canoe localize to the whole metaphase furrow during

312 mitosis 13 (green), whereas basal proteins (red) localize to the tip and stay there with

313 remodeling to cellularization furrows. With midblastula transition and onset of zygotic gene

314 expression a yet unknown zygotic gene leads to the remodeling of future subapical proteins in

315 old and new cellularization furrows and subapical domain formation is initiated.

316 Materials and Methods

317 Fly stocks and handling

318 Fly stocks used were CanoeYFP (*cno*[*CPTI000590*], Drosophila Genomics and

319 Genetic Resources, Kyoto), UASp-CherrySlam driven by maternal Gal4 (18),

320 *rap1*[*P5709*] (R. Reuter, University of Tübingen, Germany)(19) , *dizzy*(48) (R.

321 Reuter) (20), *RPII215*[*X161*] (9), *sésame* (*Hira*[*185b*]) (21); UASbazRNAi

322 (Bloomington stock # 35002), maternal triple driver MTD-Gal4 (22). As wild type

323 control *w*[*1118*] was used.

324 All fly stocks were provided by the Drosophila Stock Center, Bloomington, if not

325 stated differently. Genetic markers and annotations are described in Flybase

326 (<http://flybase.org>)(23). All crosses and cages were kept at 25°C. Germ line clones

327 were produced with the *ovo*/Flipase technique as described previously (24). *bazooka*

was depleted by overexpression of a short hairpin RNA with MTD-Gal4 during oogenesis.

Immunostainings and antibodies

Following primary antibodies were employed: mouse anti-Armadillo (1:50; N27A1, Hybridoma Center); rabbit anti-Bazooka (1:1000; A. Wodarz)(25); rabbit anti-Canoe (1:1000; (15)); mouse anti-Dlg (1:100; 4F3, Hybridoma Center); guinea pig anti-Sponge (1:1000; (26)). F-actin was stained by Phalloidin coupled to Alexa647 (Thermo Fisher). Secondary antibodies were labeled with Alexa 488, 568, 647 (Thermo Fisher). GFP tagged proteins was detected with GFP-booster coupled with Atto488 (1:500; Chromotek). DNA was stained by DAPI (0.2 µg/ml; Thermo Fisher).

Embryos were fixed by 4% formaldehyde or by heat fixation using standard methods described previously (27) and stored in methanol at -20°C. For F-actin staining with phalloidin and in the α -amanitin experiments, embryos were fixed by 8% formaldehyde and manually released from the vitelline membrane. For staining, embryos were transferred to PBT (Phosphate buffered saline (PBS) + 0.2% Tween20), washed trice for 5 min and afterwards blocked for 30–60 min in PBT+5% bovine serum (BSA). Embryos were incubated with primary antibodies in PBT+0.1% BSA overnight at 4°C or for 2–3 h at room temperature. Afterwards the embryos were washed with PBT trice for 15 min, incubated with secondary antibodies in PBT for 1–2 h at room temperature and again washed 3× with PBT for 15 min and stained with DAPI for 10 min at room temperature. The embryos were mounted in Aquapolymount (Thermo Fisher).

Injection of α -amanitin for inhibition of RNA polymerase II was conducted with a concentration of 1 mg/ml in water according to standard procedures as described before (28,29). Afterwards, the embryos were staged to reach interphase 14/15 and

fixed as described above. The vitelline membrane was manually removed prior to the staining procedure.

Imaging and Software

Imaging was performed with a Zeiss LSM780 confocal microscope equipped with an Airyscan detector unit. Fixed samples were imaged with an LCI Plan Neofluar 63×/water NA 1.3 objective. Live imaging was conducted with a Plan Neofluar 63×/oil NA 1.4 objective. Embryos for live imaging were prepared as previously described (30). Fixed samples were imaged with a frame size of 512x512 pixel (67.5×67.5 μm; 130 nm lateral pixel size) for top views and 512×200 pixel (96.4×29.4 μm; 190 nm lateral pixel size) for side views. Top views were conducted as z-stacks with a step size of 0.5 μm. Live imaging was conducted in the Airyscan mode with a frame size of 376×376 pixel (31.7×31.7 μm, 80 nm lateral pixel size). Top views were conducted as axial stacks with a step size of 0.25 μm. Orthogonal views were constructed in Fiji/ImageJ (31). Image were processed in Fiji/ ImageJ, Adobe Photoshop and Illustrator.

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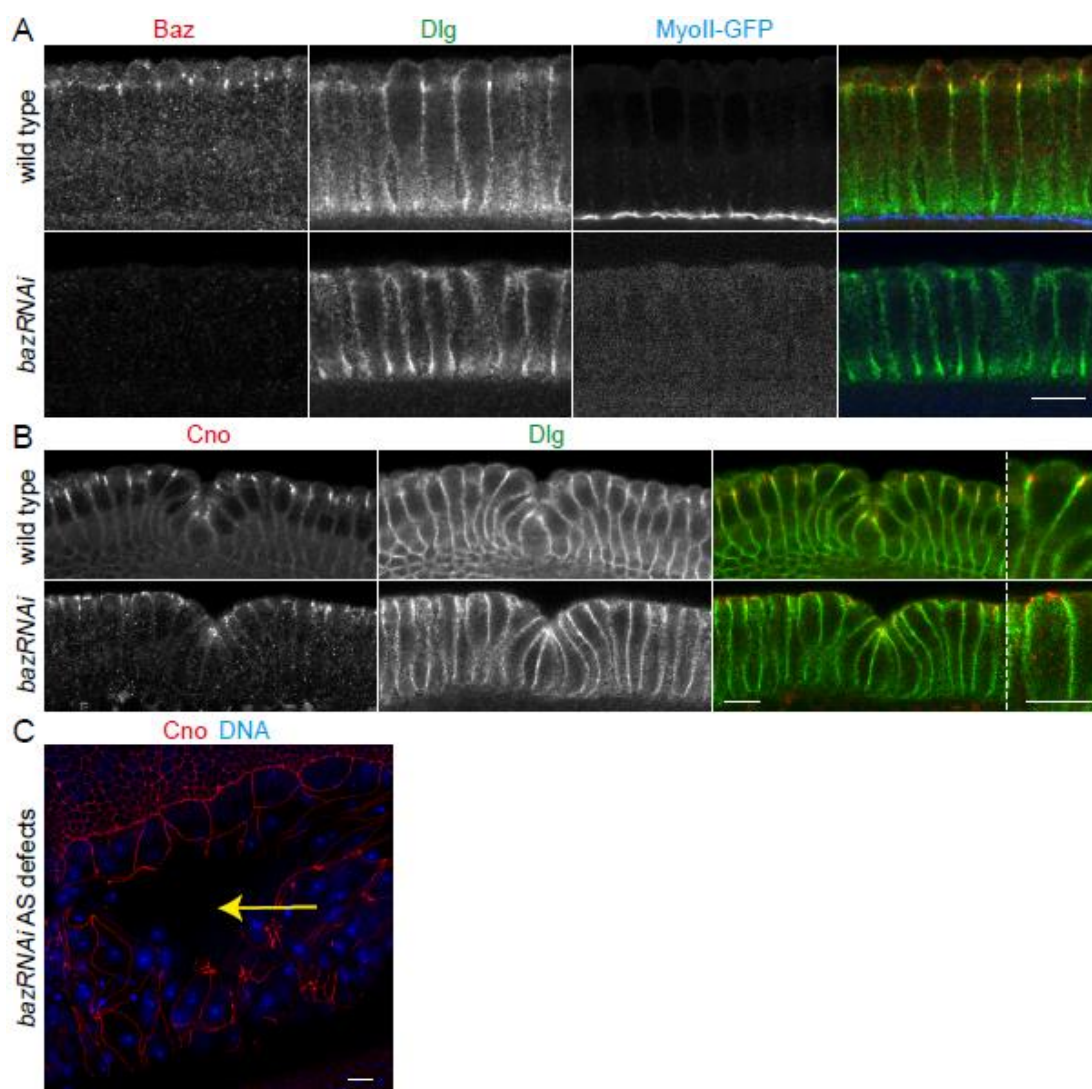
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Supplemental figures



Supplemental figure 1 Functionality of *bazooka* knock down. (A) Wild type embryos expressing MyoII-GFP and *bazRNAi* embryos fixed and stained during late cellularization against Baz (grey/red), Dlg (grey/ green) and MyoGFP (grey/ blue). MyoGFP and *bazRNAi* embryos were fixed and stained in the same tubes and imaged with same settings. (B) Fixed wild type and *bazRNAi* embryos during early gastrulation stained against Cno (grey/ red) and Dlg (grey/ green). (C) Stage 13 *bazRNAi* embryo fixed and stained against Cno (red) and DNA (blue) showing typical amnioserosa holes (yellow arrow). Scale bars 10 μ m.