ELSEVIER

Gene Expression Patterns 3 (2003) 629–634

GEP

www.elsevier.com/locate/modgep

Temporal comparison of Broad-Complex expression during eggshell-appendage patterning and morphogenesis in two *Drosophila* species with different eggshell-appendage numbers

Karen E. James, Celeste A. Berg*

Department of Genome Sciences, Box 357730, University of Washington, Seattle, WA 98195-7730, USA

Received 20 March 2003; accepted 7 May 2003

Abstract

A central question in biology is how developmental mechanisms are altered to bring about morphological evolution. Drosophilids boast a remarkable diversity in eggshell-appendage number—from as few as one to as many as nine, depending on the species. Appendage patterning in *Drosophila melanogaster* is well characterized, inviting candidate-gene-based approaches that identify the developmental mechanisms underlying Drosophilid eggshell diversity. Previous studies show that a combination of Epidermal growth factor receptor (EGFR) and TGFβ/BMP2,4 Decapentaplegic (DPP) signaling determines appendage fate in *D. melanogaster*. *Broad-Complex* expression integrates EGFR and DPP signaling and predicts future appendage position. Here we present our confocal analyses of BR-C immunofluorescence and appendage morphogenesis in *Drosophila melanogaster* (two appendages) and *Drosophila virilis* (four appendages). Our comparison suggests that differences in BR-C patterns among Drosophilids may be strongly influenced by anterior–posterior information.

Keywords: Broad-Complex; Dorsoventral patterning; Dorsal appendages; *dpp*; *Drosophila melanogaster*; *Drosophila virilis*; Epidermal growth factor receptor; Evolution; Oogenesis; *torpedo*; Transforming growth factor-β

1. Results and discussion

The *Drosophila melanogaster* eggshell and its two dorsal anterior appendages (Fig. 1A,B) are secreted by a somatic follicular epithelium that surrounds the germline cells during oogenesis (King, 1970). The eggshell appendages, or dorsal appendages, are formed when two groups of columnar follicle cells, the dorsal-appendage primordia, undergo epithelial morphogenesis late in oogenesis, creating two tubes into which is secreted the appendage chorion (J. Dorman, KEJ, and CAB, unpublished observations). Genetic analyses in *D. melanogaster* demonstrate that two major signal transduction pathways pattern the appendage primordia (for reviews, see Dobens and Raftery, 2000; Nilson and Schüpbach, 1999; van Eeden and St Johnston, 1999). The Epidermal growth factor receptor (EGFR) pathway determines the dorsoventral position of the appendages: activation of EGFR in the dorsal follicle cells triggers autocrine amplification and dorsal-midline inhibition of EGFR, resulting in two distinct dorsolateral appendage primordia (Peri et al., 1999; Sapir et al., 1998; Wasserman and Freeman, 1998; reviewed in Van Buskirk and Schüpbach, 1999). The Transforming growth factor-β/BMP2,4 homolog Decapentaplegic (DPP) pathway is required to specify the anterior–posterior position of the dorsal-appendages (Deng and Bownes, 1997; Dequier et al., 2001; Dobens et al., 2000; Twombly et al., 1996). Gain-of-function approaches indicate that a combination of Gurken/EGFR and DPP signaling are not only necessary but also sufficient to induce eggshell-appendage formation (Queenan et al., 1997; Peri and Roth, 2000).

While dorsal-appendage patterning in *D. melanogaster* is well understood, the developmental mechanism underlying appendage-number differences among the Drosophilids is unknown. Recent numerical analyses of a mathematical model (Shvartsman et al., 2002) suggest that the EGFR-feedback mechanisms that produce two dorsolateral peaks

* Corresponding author. Tel.: +206-543-1677; fax: +206-543-0754.
E-mail address: berg@gs.washington.edu (C.A. Berg).

1567-133X/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S1567-133X(03)00136-4

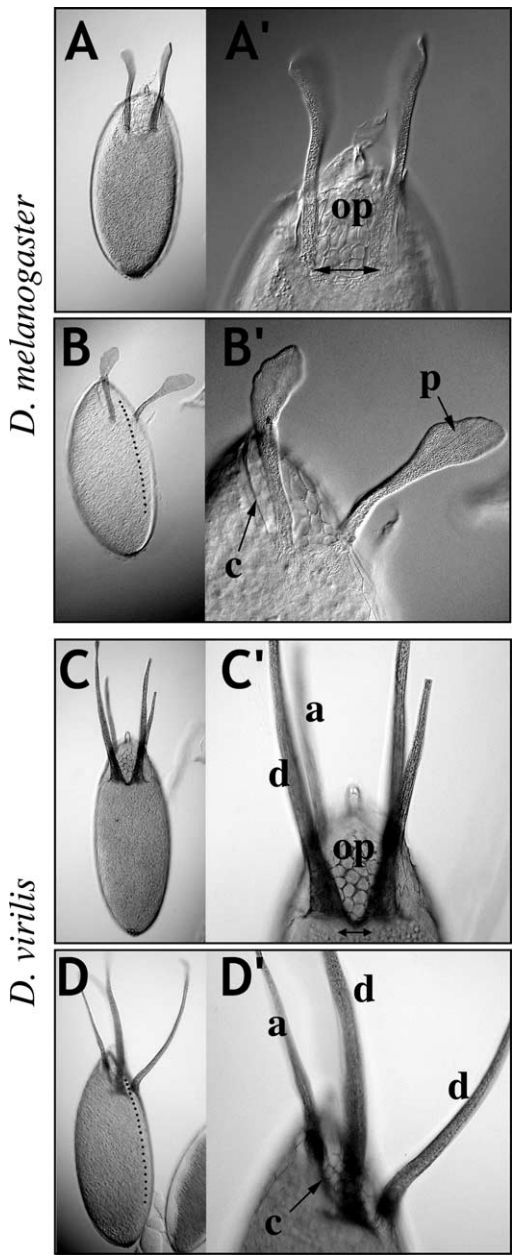

Fig. 1. Light micrographs of the eggshells of *D. melanogaster* and *D. virilis*, the end products of the developmental and morphogenetic events underlying eggshell morphology. Anterior is at the top of all panels. The dorsal midline is indicated with a dotted line in B and D. op = operculum, c = collar, p = paddle. Each pair of images (e.g., A and A′) shows a lower magnification (A) and higher magnification (A′) photomicrograph of the same egg. Dorsal (A, A′) and dorsolateral (B, B′) views of *D. melanogaster* eggshells bearing a single pair of dorsal appendages that terminate in a broad flat 'paddle'. Dorsal (C, C′) and dorsolateral (D, D′) views of *D. virilis* eggshells bearing one 'dorsal' pair of appendages (d), which reside at the posterior edge of the operculum, immediately flanking the dorsal midline. A shorter 'anterior' pair of appendages (a) resides more ventral and anterior along the collar of the operculum. The right-hand anterior appendage in D′ is out of the plane of focus. All four *D. virilis* appendages lack paddles. In both species, appendages always emerge from the edge of the operculum. The dorsal pair of *D. virilis* appendages is more closely spaced than the two *D. melanogaster* appendages (double-headed arrows in A vs. C). Overall, our observations are consistent with the published photographs and drawings of Throckmorton (1962); Patterson and Stone (1952), and Ashburner (1989).

of EGFR activity in *D. melanogaster* may be modulated to pattern multiple appendage pairs in other species.

As a first step in addressing the evolution of appendage number in vivo, we analyzed expression of Broad-Complex protein (BR-C) in egg chambers of *D. virilis* (4 appendages) and *D. melanogaster* (2 appendages). BR-C is an ideal marker because BR-C expression integrates EGFR and DPP signaling pathways during dorsal-appendage patterning in *D. melanogaster* (Fig. 2) (Deng and Bownes, 1997; Dequier et al., 2001; Peri and Roth, 2000). Furthermore, BR-C expression predicts future appendage position, in both wild-type egg chambers and appendage-patterning mutants (Deng and Bownes, 1997; Dequier et al., 2001). We reasoned that the nature of the differences, if any, in BR-C expression between the two species might shed light on the appendage-patterning process and the nature of its modulation during evolution.

1.1. The relationship between BR-C immunofluorescence and eggshell appendage morphogenesis in D. melanogaster *and* D. virilis

Beginning around stage 10a of oogenesis in *D. melanogaster*, BR-C expression comes under the control of EGFR and DPP signaling (Deng and Bownes, 1997; Dequier et al., 2001; Peri and Roth, 2000). Since this BR-C expression pattern changes shape over time during appendage patterning and morphogenesis (Tzolovsky et al., 1999), we conducted a spatiotemporal comparison, examining several samples from each of five stages spanning appendage patterning and morphogenesis (Fig. 3).

At stage 10b in *D. virilis* and stage 10a in *D. melanogaster*, BR-C immunofluorescence first disappears from a dorsal anterior region and then begins to intensify in two anterior dorsolateral populations (Fig. 3A, F). Because of the difference in timing of this BR-C patterning event in stage 10, we reasoned that traditional egg-chamber staging criteria (outlined in Section 2) might cause inappropriate comparisons. Therefore, we compared BR-C expression relative to the onset of appendage morphogenesis, which, in *D. melanogaster*, is characterized by the apical constriction of the appendage-forming follicle cells during stage 10b/11 (J. Dorman and CAB, unpublished observations). This event can be visualized using an E-Cadherin antibody, which marks the apical footprints (the zonula adherens) of follicle cells (James et al., 2002; Niewiadomska et al., 1999; J. Dorman and CAB, unpublished observations). Using this method, we found that morphogenesis begins later in *D. virilis* (stage 11/12) than it does in *D. melanogaster* (stage 10b/11), and the anterior pair of *D. virilis* appendage primordia begins morphogenesis slightly later than the dorsal pair (Fig. 3H).

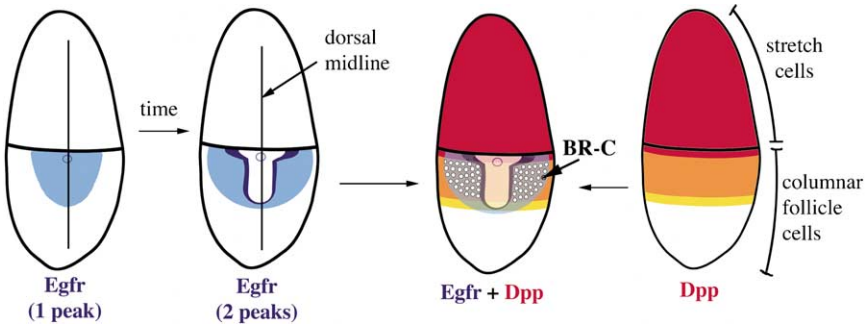

Fig. 2. DPP and EGFR signals coordinately specify two dorsolateral appendage primordia, marked by high levels of BR-C expression (white dots), in *D. melanogaster*. Each drawing depicts a dorsal view of the follicular epithelium. Anterior is at the top. Dorsal activation of EGFR (light blue) by Gurken, localized over the oocyte nucleus (circle on midline), triggers a secondary amplification and midline inhibition of the dorsal EGFR-signaling domain. This process produces two dorsolateral regions, or 'peaks', of high EGFR activity (blue) separated by a dorsal minimum (white). Phosphorylated MAP-kinase immunoreactivity, a readout of EGFR signaling, splits into these two domains, with especially high levels of signal (dark blue) in two brows, or hinges, adjacent to the dorsal midline (Peri et al., 1999). DPP (red) is expressed in two subsets of follicle cells that lie anterior to the appendage primordia: the nurse-cell-associated 'stretch' cells, a squamous layer of follicle cells covering the nurse cell compartment of the egg chamber, and the leading edge of the centripetally migrating follicle cells at the anterior edge of the columnar epithelium (Dobens et al., 2000; Twombly et al., 1996). This row of columnar follicle cells will migrate centripetally between the oocyte and nurse cells and lie buried between these germ cells from stage 10b on. Red, orange, and yellow regions depict the DPP morphogen gradient within the columnar epithelium. High levels of DPP signaling (red) repress BR-C expression, while moderate levels of DPP activate BR-C expression.

1.2. General appendage patterning and morphogenetic mechanisms are conserved between D. melanogaster *and* D. virilis

It was formally possible that *D. virilis* eggshell appendages are patterned and formed via a mechanism that differs significantly from appendage development in *D. melanogaster*. Peri et al. (1999) observed, however, that *gurken* mRNA localization is similar in the two species, suggesting that appendage patterning is initiated via the same mechanism in both species. In addition, we found that high BR-C expression correlates with appendage morphogenesis in both species (Fig. 3). Furthermore, the morphogenetic events of appendage formation in *D. melanogaster*, such as apical constriction and other shape changes (J. Dorman and CAB, unpublished observations), are conserved in *D. virilis* (Fig. 3). Together, these observations indicate that the general characteristics of BR-C expression and morphogenesis observed in *D. melanogaster* are conserved in all four *D. virilis* appendage primordia.

1.3. Spatiotemporal comparison of BR-C immunofluorescence during eggshell appendage patterning and morphogenesis in D. melanogaster *vs.* D. virilis

Despite this gross conservation of the process, the shape of the BR-C expression pattern is quite different in the two species (Figs. 3 and 4). In *D. melanogaster*, BR-C is up-regulated dorsolaterally and down-regulated on the dorsal midline in an EGFR-dependent manner (Deng and Bownes, 1997; Dequier et al., 2001; Peri et al., 1999; Tzolovsky et al., 1999). Surprisingly, we observed that high BR-C expression in *D. virilis* begins as two dorsolateral patches but does *not* split into four distinct patches (Figs. 3 and 4). Analysis of additional EGFR-responsive markers in the two species will be necessary to test whether EGFR signaling is inhibited between the dorsal and anterior appendage primordia in *D. virilis*.

The BR-C pattern in *D. virilis* is larger and spreads more laterally than in *D. melanogaster*, and the dorsal anterior region of high BR-C expression in *D. virilis* also lies more posterior than in *D. melanogaster* (Figs. 3 and 4). This *D. virilis* BR-C pattern bears a striking resemblance to a micrograph published by Deng and Bownes (1997), which shows BR-C expression in a *D. melanogaster* egg chamber in which *dpp* has been over-expressed in all columnar follicle cells using the GAL4/UAS system. Significantly, more than two and sometimes as many as six dorsal appendages adorn the eggshells of *dpp*-over-expression flies (Deng and Bownes, 1997; Dequier et al., 2001; Twombly et al., 1996). In contrast, increasing the dosage of EGFR ligands or ectopically activating EGFR in *D. melanogaster* does not generate a *D. virilis*-like BR-C pattern nor induce ectopic appendages (Deng and Bownes, 1997; Ghiglione et al., 2002; Pai et al., 2000; Sapir et al., 1998).

Morphological evolution results from the natural selection of genetic changes that affect development. Our studies begin to examine the molecular mechanisms underlying the evolution of Drosophilid eggshell morphology. We anticipate that future analyses of additional pathway-specific markers will help clarify the respective contributions of the DPP and EGFR pathways to the specification of appendage number. Furthermore, we hope that future analyses of candidate genes will ultimately discover the mechanisms underlying the evolution of different Drosophilid eggshell-appendage numbers.

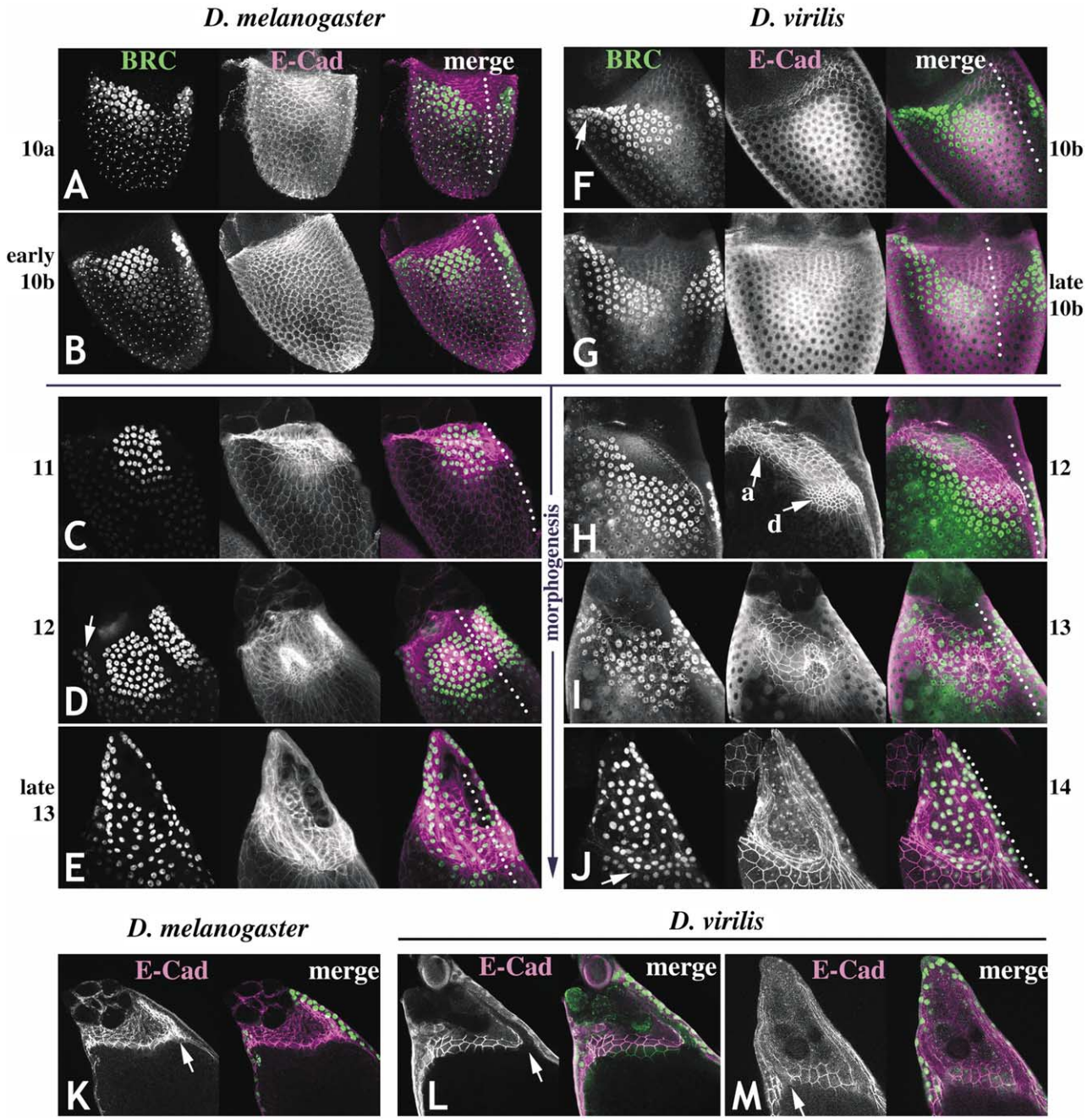

Fig. 3. Temporal comparison of Broad-Complex (BR-C) protein expression and dorsal-appendage morphogenesis in *D. melanogaster* (A–E, and K) and *D. virilis* (F–J, and L, M). A–J) Confocal projections of the dorsal-appendage primordia. Each panel of three images shows a dorsolateral view of the same egg chamber stained with anti-BR-C (green), anti-E-Cadherin (purple, for morphology), and the merged image. The dorsal midline is indicated with a white dotted line in each merged image. The numbers to the right and left of each panel designate developmental stage based on the relative volume of oocyte to nurse cells (see Section 2); the panels are aligned, however, to facilitate comparison based on timing relative to the onset of dorsal-appendage morphogenesis. In both species, BR-C expression is first excluded from a dorsal anterior patch (not shown) and then intensifies in two dorsolateral patches (A, B, F, G). The intensity and number of high-BR-C-expressing cells achieves a maximum at approximately late stage 10b in *D. melanogaster* and stage 11 in *D. virilis*. During intensification, the edges of the BR-C pattern become more refined in both species (B, G), and differences in the size and spatial conformation of the BR-C patterns begin to emerge. The dorsal-midline down-regulation of BR-C is 5 cells wide in *D. melanogaster*, and only 3 cells wide in *D. virilis*. This difference correlates with the observed distance between mature appendages (Fig. 1). In *D. melanogaster*, BR-C is excluded from an anterior band ranging from one row laterally to three rows directly adjacent to the dorsal-midline region of exclusion. In *D. virilis*, the anterior region of exclusion is dramatically deeper near the midline, ranging from one row laterally to approximately eight rows near the dorsal-midline region of exclusion. Correspondingly, the region of high BR-C expression near the dorsal midline in *D. virilis* is also pushed posteriorward. As BR-C expression in *D. virilis* continues to intensify (shown more clearly in Fig. 4C), the ventro-anterior 'tails' of BR-C expression become thicker and more intensely fluorescent. The domain of high BR-C expression narrows slightly between the original dorsolateral patch and the extended tail, creating a subtle 'dumbbell' shape, but, importantly, the intensity of immunofluorescence in this region still remains high compared to the main-body follicle cells. As morphogenesis begins, the two dorsolateral regions of BR-C expression in *D. melanogaster* become more

2. Materials and methods

2.1. Fly stocks, immunofluorescence, developmental staging, and microscopy

We employed our lab strain Canton S to represent wild-type *D. melanogaster* and ordered wild-type *D. virilis* from the Tucson *Drosophila* Species Stock center. We fixed ovaries with 4% paraformaldehyde in phosphate-buffered saline (PBS) and washed them in PBS/0.1% Triton X-100 (with 5% normal goat serum added during blocking and antibody incubations). For BR-C/E-CAD co-immunofluorescence, we used the following antisera: mouse monoclonal anti-Broad-core antibody '25E9' (Emery et al., 1994) diluted to 1:100 and used at least once previously (re-using the antibody was especially critical to achieve staining in *D. virilis*); rat monoclonal anti-*Drosophila*-E-Cadherin 'D-CAD2' (1:50) (Oda et al., 1994). We detected the primary antibodies with AlexaFluor488 goat anti-mouse and AlexaFluor568 goat anti-rat (both diluted to 1:100) secondary antibodies from Molecular Probes. We examined labeled ovaries using a Leica Spectral Confocal Microscope. We employed NIH Image and Adobe Photoshop to analyze and process confocal images.

To categorize the developmental stages of *D. melanogaster* and *D. virilis* egg chambers, we used the following criteria. In stage 10a, the oocyte takes up half the volume of the egg chamber; stage-10b egg chambers are larger than 10a and have initiated centripetal migration. In stage-11 egg chambers, the oocyte is larger than the nurse chamber due to the onset of nurse cell dumping, while during stage 12, dumping is completed but 15 nurse cells are still visible. Stage-13 egg chambers have a decreased nurse-cell number due to apoptosis, and stage-14 egg chambers have no nurse-cell nuclei remaining. These criteria are based on standard staging principles (King, 1970; Spradling, 1993) except that the appearance of dorsal appendages was not considered.

2.2. Unbiased comparison of BR-C using immunofluorescence normalization and an Image J look-up table

In both *D. melanogaster* and *D. virilis*, expression of BR-C protein lingers at low levels in the main-body follicle cells (Fig. 3; Tzolovsky et al., 1999). Therefore, three major categories of BR-C protein expression levels exist in

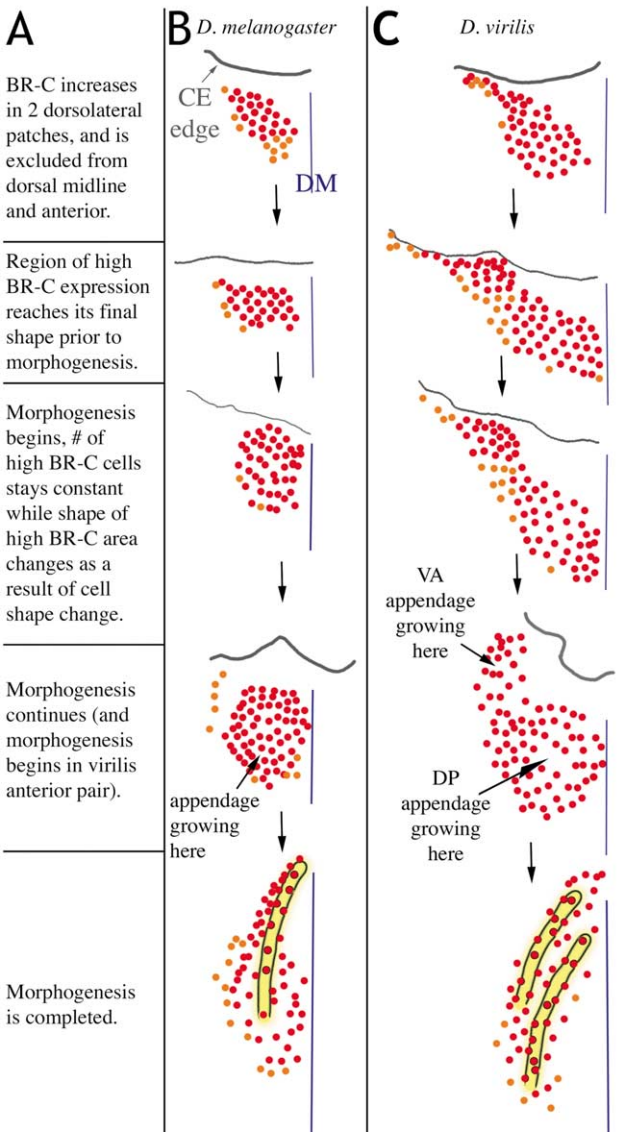

Fig. 4. Comparison of BR-C expression profiles in *D. melanogaster* and *D. virilis*. (A) Description of morphogenetic events and BR-C expression-pattern changes. Dot schematics of BR-C expression patterns in *D. melanogaster* (B) and *D. virilis* (C). Anterior is up, and each schematic shows the *left half only* of the BR-C pattern at the anterior region of the egg chamber. DM = dorsal midline (blue line), CE edge = anterior edge of columnar epithelium (gray line). VA appendage = ventral-anterior appendage; DP appendage = dorsal-posterior appendage. Red dots mark cells expressing high levels of BR-C, while orange dots mark moderate levels of BR-C expression. Indicated on the schematics of the final time points in B and C are the rough positions of the appendages, in pale yellow, which lie beneath the BR-C-expressing cells.

circular (C), perhaps due to the cell-shape changes that occur at this time. In *D. virilis*, cell-shape changes also begin to alter the original BR-C pattern (H), making the boundaries and areas of the pattern less even. Notably in the stage-12 *D. virilis* egg chamber shown in H, apical constriction has occurred in the dorsal set of appendages (d) but not yet in the anterior set (a). As morphogenesis progresses in both species (D, E and I, J), BR-C expression remains high in the appendage-forming cells. In *D. melanogaster*, some of the cells that are lateral to the appendage-forming groups express moderate levels of BR-C (arrow in D). This expression is modest, however, compared to the 'tails' of the *D. virilis* BR-C regions, and these cells appear to be physically distinct from the appendage-forming follicle cells (not shown) and may form the collar surrounding the operculum. Indeed, in *D. virilis*, this medium level of BR-C immunofluorescence can be seen in the cells forming the collar in a more mature egg chamber (arrow in J). (K–M) Optical cross-sections through side-views of developing dorsal appendages (the base of the relevant appendage is indicated with an arrow in each panel). Projections of several confocal sections through each appendage confirm that BR-C is expressed in the cells forming *D. melanogaster* appendages (K) and both 'dorsal' (L) and 'anterior' (M) *D. virilis* appendages.

the columnar follicle cells: very low in dorsal midline and anterior rows, medium in the main-body follicle cells, and high in the anterior dorsolateral follicle cells. The boundary between high and medium BR-C expression is subtle and graded.

To compare BR-C expression levels in the two species accurately, we created the Fig. 4 expression profile as follows. We first selected representative dorsolateral or lateral-view images from each stage. Lateral views were especially helpful for visualizing the full extent of the *D. virilis* BR-C pattern. We then normalized the immunofluorescence signal in each individual image using the Adobe Photoshop command 'adjust auto levels'. We imported this image into the public Java image-processing program, Image J (created by Wayne Rasband; http://rsb.info.nih.gov/ij/download.html), and pseudo-colored the image using the Spectrum look-up table (LUT). Pseudo-colorization let us more easily detect the differences between high, medium, and low levels of BR-C immunofluorescence. We re-opened the image in Adobe Photoshop and used the paintbrush tool to draw red dots on the BR-C high nuclei and orange dots on the BR-C medium nuclei. Finally, we added lines for orientation (dorsal midline and anterior edge of epithelium) and then isolated the Photoshop layer containing the dots and lines in a new file.

Acknowledgements

We thank members of the Berg and Ruohola-Baker laboratories, Francesca Peri and Siegfried Roth for technical advice, insight, and helpful discussions. Jennie Dorman's characterization of wild-type appendage formation in *D. melanogaster* provided the morphogenetic context crucial for our analysis. In addition, we thank Gregory Guild and Tadashi Uemura for sending antibodies, and Pernille Rørth for protocol advice. We thank Ellen Ward for critical comments on the manuscript. This work was supported by National Science Foundation grant IBN-9983207.

References

Ashburner, M., 1989. *Drosophila*, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 214–215.

Deng, W.M., Bownes, M., 1997. Two signalling pathways specify localised expression of the Broad-Complex in *Drosophila* eggshell patterning and morphogenesis. Development 124, 4639–4647.

Dequier, E., Souid, S., Pal, M., Maroy, P., Lepesant, J.A., Yanicostas, C., 2001. Top-DER- and DPP-dependent requirements for the *Drosophila fos/kayak* gene in follicular epithelium morphogenesis. Mech. Dev. 106, 47–60.

Dobens, L.L., Raftery, L.A., 2000. Integration of epithelial patterning and morphogenesis in *Drosophila* ovarian follicle cells. Dev. Dyn. 218, 80–93.

Dobens, L.L., Peterson, J.S., Treisman, J., Raftery, L.A., 2000. *Drosophila bunched* integrates opposing DPP and EGF signals to set the operculum boundary. Development 127, 745–754.

Emery, I.F., Bedian, V., Guild, G.M., 1994. Differential expression of Broad-Complex transcription factors may forecast tissue-specific developmental fates during *Drosophila* metamorphosis. Development 120, 3275–3287.

Ghiglione, C., Bach, E.A., Paraiso, Y., Carraway, K.L., Noselli, S. 3rd, Perrimon, N., 2002. Mechanism of activation of the *Drosophila* EGF Receptor by the TGF-α ligand Gurken during oogenesis. Development 129, 175–186.

James, K.E., Dorman, J.B., Berg, C.A., 2002. Mosaic analyses reveal the function of *Drosophila* RAS in embryonic dorsoventral patterning and dorsal follicle-cell morphogenesis. Development 129, 2209–2222.

King, R.C., 1970. Ovarian Development in *Drosophila melanogaster*, Academic Press, New York.

Niewiadomska, P., Godt, D., Tepass, U., 1999. DE-Cadherin is required for intercellular motility during *Drosophila* oogenesis. J. Cell Biol. 144, 533–547.

Nilson, L.A., Schüpbach, T., 1999. EGF receptor signaling in *Drosophila* oogenesis. Curr. Top. Dev. Biol. 44, 203–243.

Oda, H., Uemura, T., Harada, Y., Iwai, Y., Takeichi, M., 1994. A *Drosophila* homolog of Cadherin associated with Armadillo and essential for embryonic cell–cell adhesion. Dev. Biol. 165, 716–726.

Pai, L.M., Barcelo, G., Schüpbach, T., 2000. D-cbl, a negative regulator of the EGFR pathway, is required for dorsoventral patterning in *Drosophila* oogenesis. Cell 103, 51–61.

Patterson, J.T., Stone, W.S., 1952. Evolution in the Genus *Drosophila*, The Macmillan Company, New York.

Peri, F., Roth, S., 2000. Combined activities of Gurken and Decapentaplegic specify dorsal chorion structures of the *Drosophila* egg. Development 127, 841–850.

Peri, F., Bokel, C., Roth, S., 1999. Local Gurken signaling and dynamic MAPK activation during *Drosophila* oogenesis. Mech. Dev. 81, 75–88.

Queenan, A.M., Ghabrial, A., Schüpbach, T., 1997. Ectopic activation of *torpedo*/EGFR, a *Drosophila* receptor tyrosine kinase, dorsalizes both the eggshell and the embryo. Development 124, 3871–3880.

Sapir, A., Schweitzer, R., Shilo, B.Z., 1998. Sequential activation of the EGF receptor pathway during *Drosophila* oogenesis establishes the dorsoventral axis. Development 125, 191–200.

Shvartsman, S.Y., Muratov, C.B., Lauffenburger, D.A., 2002. Modeling and computational analysis of EGF receptor-mediated cell communication in *Drosophila* oogenesis. Development 129, 2577–2589.

Spradling, A.C., 1993. Developmental genetics of oogenesis, In: Bate, M., Martinez-Arias, A. (Eds.), The Development of *Drosophila melanogaster*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 1–70.

Throckmorton, L., 1962. The problem of phylogeny in the genus *Drosophila*. In: Wheeler, M.R., (Ed.), Studies in Genetics, vol. II. University of Texas Publication, Austin, TX, pp. 207–343.

Twombly, V., Blackman, R.K., Jin, H., Graff, J.M., Padgett, R.W., Gelbart, W.M., 1996. The TGF-β signaling pathway is essential for *Drosophila* oogenesis. Development 122, 1555–1565.

Tzolovsky, G., Deng, W.M., Schlitt, T., Bownes, M., 1999. The function of the Broad-Complex during *Drosophila melanogaster* oogenesis. Genetics 153, 1371–1383.

Van Buskirk, C., Schüpbach, T., 1999. Versatility in signalling: multiple responses to EGF receptor activation during *Drosophila* oogenesis. Trends Cell Biol. 9, 1–4.

van Eeden, F., St Johnston, D., 1999. The polarisation of the anterior-posterior and dorsal-ventral axes during *Drosophila* oogenesis. Curr. Opin. Genet. Dev. 9, 396–404.

Wasserman, J.D., Freeman, M., 1998. An autoregulatory cascade of EGF-receptor signaling patterns the *Drosophila* egg. Cell 95, 355–364.