Abstract

Extraembryonic development is familiar to mouse researchers, but the term is largely unknown among insect developmental geneticists. This is not surprising, as the model system Drosophila melanogaster has an extremely reduced extraembryonic component, the amnioserosa. In contrast, most insects retain the ancestral complement of two distinct extraembryonic membranes, amnion and serosa. These membranes are involved in several key morphogenetic events at specific developmental stages. The events of anatrepsis and katatrepsis—collectively referred to as blastokinesis—are specific to hemimetabolous insects. Corresponding events in holometabolous insects are simplified and lack formal names. All insects retain dorsal closure, which has been well studied in Drosophila. This review aims to resurrect both the terminology and awareness of insect extraembryonic development—which were last common currency in the late nineteenth and early twentieth centuries—as a number of recent studies have identified essential components of these events, through RNA interference of developmental genes and ectopic hormonal treatments. As much remains unknown, this topic offers opportunities for research on tissue specification, the regulation of cell shape changes and tissue interactions during morphogenesis, tracing the origins and final fates of cell and tissue lineages, and ascertaining the membranes’ functions between morphogenetic events.

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Introduction

Insect extraembryonic development encompasses all aspects of embryogenesis involving the amniotic and serosal membranes. In this review I will focus primarily on the role of the membranes in morphogenetic events, specifically the movements associated with membrane formation—anatrepsis—and their last acts—katatrepsis and dorsal closure—prior to their demise. These movements are particularly well developed in hemimetabolous insects (those with incomplete metamorphosis) and are collectively known as blastokinesis. Extraembryonic membrane ontogeny in the holometabolous insects (those with complete metamorphosis: embryonic, larval, pupal, and adult stages) includes a subset of these events.

The occurrence of blastokinesis was last reviewed in detail 35 years ago by Anderson (1972a), who regarded it as an “additional complication” (p. 130) to embryogenesis in some insect orders. Yet extraembryonic development is relevant to the development of all insects. Researchers who are currently familiar with this fact include embryological endocrinologists (e.g., Truman and Riddiford, 1999) and classical morphologists (extraembryonic membranes even feature in the logo of the Arthropodan Embryological Society of Japan). Meanwhile, Anderson’s view is still prevalent among developmental geneticists. Blastokinesis does not occur in the model organism Drosophila melanogaster, the embryos of which have very reduced extraembryonic tissue (although this tissue is still essential for development). As a consequence, many researchers are either unaware of the phenomena of extraembryonic development or have glossed over them in their own research species. This is reflected in searching the PubMed database of biomedical and life science articles (http://www.ncbi.nlm.nih.gov), where there are thousands of articles on “insect embryo” generally but only a handful specifically for “blastokinesis” or “insect extraembryonic development.” As I hope to show here, extraembryonic development is fascinating...
and worthy of researchers’ attention in its own right for study of a multitude of developmental processes.

This review is two-pronged: it surveys manifestations of blastokinesis, and it summarizes relevant functional data on these movements. To provide adequate context, I begin with a background account of the evolution and possible functional roles of the membranes. To introduce the nature and complexity of the morphogenetic events, blastokinesis is first described in detail in a representative species. This is followed by the comparative survey of extraembryonic morphogenesis across the insects. I then assess the relationship of the membranes to the execution of these movements in different species. Lastly, I present recent data from perturbation studies that elucidate some of the molecular components underlying extraembryonic morphogenesis, and identify possible avenues for further research.

**Phylogeny of extraembryonic membrane elaboration and reduction**

Extraembryonic cells or tissues are common features of embryogenesis in animals. This material has become elaborated in the insects to comprise two distinct membranes, with each possessing a distinct topography and role. The inner membrane, the amnion, envelops the ventral side of the developing embryo, creating the fluid-filled amniotic cavity. The outer membrane, the serosa, lies just under the chorion (eggshell), and surrounds embryo, amnion, and yolk (Fig. 1). Presence of these two membranes is regarded as a synapomorphy (shared, derived region; gn, gnathal (mouthpart) region; h, head; ser, serosa; t1 in the sagittal view. Abbreviations: a, antenna; am, amnion; ab, abdominal region; gn, gnathal (mouthpart) region; h, head; ser, serosa; t1–3, thoracic segments/legs 1–3. The position of the embryo is modeled on the immersed limb, as seen in the milkweed bug *Oncopeltus fasciatus* (see also Fig. 3, including the labeling of axes).

![Schematic cartoons of a germ band stage embryo, illustrating the positions of the serosa (blue) and amnion (orange) with respect to the embryo (grey) and yolk (yellow)](image)

Serosa and amnion can be considered standard inventory of the insect embryo. The absence of one or the other in apterygote insects is probably indicative of their phylogenetic development, whereas deviations, as they occur in holometabolous insects, represent presumably environmental adjustments. (Dorn, 1976)

As implied by the quotation, the membranes arose within the lineage of apterygote (primitively wingless) insects, perhaps progressively. Among non-insect hexapods (see phylogeny in Fig. 2), Collembola and Protura (springtails and allies) possess a serosa and a structure known as the primary dorsal organ (see Box 1), but they lack two distinct membranes (Anderson, 1973, p. 205; Jura, 1972; Machida, 2006; Uemiya and Ando, 1991). Similarly the Diplura (two-pronged bristletails) have a single, serosal covering over the yolk, though there is also later production of additional extraembryonic tissue, which has been termed “amnion” (Ikeda and Machida, 2001). Within the true insects, the amnion is prefigured by the pro-amniotic/pro-serosal distinction in archaeanognathan (bristletail) embryos (Heymons and Heymons, 1905; Machida et al., 1994; see Box 1). An amnion proper is first seen in the Thysanura (firebrats; Anderson, 1972a, pp. 204–205; Jura, 1972, pp. 80–82). However the thysanuran amnion does not form a complete membrane but leaves a persistently open amniotic cavity (Heymons and Heymons, 1905; Woodland, 1957). Complete amnions seem restricted to the pterygote (winged) insects, although the apterygote species survey supporting this claim is limited.

As Dorn (1976) also indicates, there are deviations from possession of two extraembryonic membranes in some holometabolous insect species (see phylogeny in Fig. 2). Here, the lack of a full membrane complement is a derived, secondary condition. Wholesale changes in embryogenesis—such as rapid development from a large embryonic rudiment (relative to egg size)—correlate with a reduction in extraembryonic tissue (Anderson, 1972a; Roth, 2004). In some lower Hymenoptera (sawflies), the amnion forms but degenerates very early in development and may be fragmentary (Ivanova-Kasas, 1959; Shafiq, 1954). Aporcratan (higher) Hymenoptera (bees, ants, and wasps) usually lack an amnion, or have only a temporary amniotic vestige that covers the yolk rather than the embryo (Anderson, 1972b; Bull, 1982; Fleig and Sander, 1988). Cyclorrhaphous (higher) Diptera (true flies), including the fruit fly *Drosophila melanogaster*, lack distinct amniotic and serosal membranes, retaining a single membrane, the amnioserosa, which also covers only the yolk (Anderson, 1972b; Schmidt-Ott, 2000).

**Proposed functions of the extraembryonic membranes**

Given the conserved presence of the extraembryonic membranes in most insects, why do they exist? Their designation as extraembryonic signifies that the amnion and the serosa do not directly contribute to the final form of the body. What function do they fulfill to necessitate their temporary existence?

Such questions have produced several suggestions. Because the membranes surround the embryo, a general protective function has been ascribed (Dorn, 1976; Osato and Helms, 1972; Zeh et al., 1989). Specifically, the fluid-filled amniotic cavity may cushion the embryo (Anderson, 1972a). The outer serosa can effect innate immune system responses to wounding or infection (Chen et al., 2000) or process environmental toxins (Bergner-Twelbeck et al., 2003). In some species, the amnion or the serosa or both are involved in cuticle production (Cobben, 1968; Dorn, 1976; Heming, 2003; Mellanby, 1936). Cuticle
secreted by the amnion can contribute to the embryonic cuticle, implicating the amnion in effecting embryonic development *per se* (Dorn, 1976). Serosal cuticle can augment or supplant protection provided by the chorion (Chen et al., 2000; Church and Rempel, 1971; Kobayashi, 1998; Miura et al., 2003; Rakshpal, 1962; Slifer, 1932), but this role is not universally applicable (Lamer and Dorn, 2001). The serosa may also have a role in water regulation (Cobben, 1968; Mori, 1972; Slifer, 1932; references cited in Dorn, 1976 and Zeh et al., 1989). Both amnion and serosa have also been implicated in yolk catabolism and active transport to sequester resulting metabolic waste, containing it within the amniotic cavity or in the space external to the serosa (Dorn, 1976, and references therein; Heming, 2003; Lamer and Dorn, 2001). Lastly, in some insect species with specialized oviposition sites, inflation of the serosa plays an active role in hatching from the egg at the end of embryogenesis (described in Sander, 1976).

However, current role does not equate with original cause of construction (i.e., insect embryos did not develop elaborate membrane covers because they would fulfill useful functions once established). An extraembryonic epithelium occurs in the eggs of many taxa outside the insects (Anderson, 1972a; Chipman et al., 2004; Wheeler, 1893; Wolff and Scholtz, 2002). Thus cellularization over the yolk surface may be a general phenomenon that simply produces more tissue than that which becomes the embryo proper. Similarly, early speculation on the...
origins of the amnion—being the more specialized and unique of the two membranes—explains its creation as a byproduct of physical forces during development. Wheeler (1893) suggested that "local induplication of the blastoderm due to rapid proliferation of a single layer of cells" could produce a fold of tissue over the rest of the blastoderm, which could then develop into a complete membrane. Alternatively, he suggested that the fluid-filled amniotic cavity might result from "peripheral and external resistance" to growth of the embryo, making the amnion analogous to a blister, cushioning the embryo from the inner surface of the eggshell! However, as described below, not all amnions form from folds or abut the eggshell, and thus different mechanical reasons would need to account for different methods of amnion formation and different topographies.

Despite over 100 years of entomological research since Wheeler's suggestions, the origins and current roles of these membranes remain uncertain, particularly for the amnion (Anderson, 1972a; Sander, 1976). Ultimately, the ancestral impetus for formation of the amnion and the serosa simply may have been the consequence of physical forces (whatever their nature) that occur during embryogenesis. Once formed, there are clearly a number of useful functions that they may perform in extant insects. Indeed, it has been observed that in each of the great bilaterian animal groups of the deuterostomes and protostomes, it is the group that has developed an amniotic cavity—the amniote vertebrates (mammals and reptiles) and the insects, respectively—that has been the most successful on land with respect to species number and range of colonized habitats (Grimaldi and Engel, 2005; Laurin and Gauthier, 1996; Zeh et al., 1989). Although analogous rather than deriving from a common ancestral structure, the amnions of vertebrates and insects seem to fulfill similar protective and metabolic functions. Thus convergence on the possession of an amnion in the egg correlates with an evolutionarily successful life history strategy—providing additional motivation for further research on this extraembryonic structure.

This introduction to insect extraembryonic membranes has dealt with their presence or absence in various taxonomic groups, and roles they may play given the arrangement of inner amnion and outer serosa. However, this topography only applies for a fraction of total developmental time of the insect egg. The amnion and the serosa are far from static structures, with the developing and mature membranes involved in extensive movements during embryogenesis in most species. Further, in some insects the membranes have become necessary for the execution of these movements. Details of membrane formation and movements, and the possible functions ascribed to these, will be discussed in the following sections.

A comparative survey of blastokinesis

“Blastokinesis, or the turning of the partially formed embryo in the egg, is a phenomenon familiar to all students of insect development.” (Slifer, 1932)

The position of the embryo relative to the yolk changes as tissues move during embryogenesis. Collectively these movements are referred to as blastokinesis. Blastokinesis varies between insects in extent and type of movements, and in the roles of different tissues in effecting the movements. Yet despite the permutations, the extraembryonic membranes are necessary for some and are inextricably involved in all of these movements. Following an introductory example of blastokinesis in one species, this comparative account reviews some of the older literature—as the opening quote by Slifer (1932) is no longer true—and thereby presents the widespread diversity of acrobatics performed within different insect eggs.

An introductory example: Blastokinesis in the milkweed bug

Here I present an overview of the movements in the milkweed bug Oncopeltus fasciatus (order Hemiptera, suborder Heteroptera: the "true bugs"). Oncopeltus is a suitable starting point as its blastokinetic movements are both complex and representative of several insect orders. Secondly, because Oncopeltus is sensitive to targeted gene knockdown by RNA interference (RNAi; Hughes and Kaufman, 2000; Liu and Kaufman, 2004), it is used increasingly as a hemimetabolous model species in comparative functional studies. Consequently, several recent experimental findings on the molecular basis of blastokinesis are from this species (see ‘How does it occur? Insights from experimental data’, below). The account of blastokinesis presented here (from Panfilio et al., 2006, and unpublished observations) corroborates the classic description of Butt (1949).

Broadly, blastokinesis encompasses early entry and later exit of embryo from yolk, termed anatrepsis and katatrepsis, respectively. Compared to other developmental stages, both of these events occur quickly. In Oncopeltus each event may take as little as 1% of development (2 h at 25 °C). The embryo, amnion, and serosa are all involved (Fig. 3).

The early, cellularized blastoderm consists of a continuous cell layer over the egg surface. Around 22% development, anatrepsis proceeds by invagination of tissue into the yolk at the posterior of the egg (Fig. 3a). This is immersion anatrepsis (further description in the following subsections on anatrepsis), and the invaginating tissue can be likened to a sock being pulled through its own opening. The tissue that remains on the surface, and spreads to fully envelop the yolk and internalized tissue, is the serosa. The invaginating tissue is the germ rudiment. Although the serosa and germ rudiment arise from a continuous sheet of tissue, after invagination the latter becomes isolated in the yolk and separated from the serosa. The germ rudiment differentiates into the amnion and embryo, such that one wall of the ‘sock’ is the embryo while the amnion comprises the other wall over the ventral side of the embryo. The embryo remains in this position during the next quarter of development—as it extends in length and then retracts, and during segmentation and appendage formation (Figs. 1, 3b).

Halfway through (50%) development, katatrepsis reverses this topography. The sock-like germ rudiment tissue (elaborated into amnion and embryo with appendages, but still a continuous bag of tissue) everts. Here "eversion" is used in the sense of turning outward (embryo and amnion), and of turning inside out.
Fig. 3. Blastokinesis during *Oncopeltus* development illustrated by schematic cartoons with accompanying nuclear stainings: (a) immersion anatrepsis, (b) extended germband stage, (c) early katatrepsis, (d) mid katatrepsis, (e) late katatrepsis. The serosa has mostly been removed in the light micrograph in panel a, such that the yolk nuclei and germ rudiment are visible. The embryo is partially visible in panels b, c where it is not deep within the yolk. Images are in lateral aspect. Orientation: egg-anterior is left and dorsal is up. Micrographs are a single focal depth (a) and confocal projections (b–e) of fluorescent nuclear staining. Schematic color coding: blue, serosa; orange, amnion; grey, embryo. Black arrows in cartoons indicate the direction of motion; white arrowheads in micrographs demarcate the amnion–serosa boundary. Abbreviations: A, P, D, and V, anterior, posterior, dorsal, and ventral axes of egg; A′, P′, and V′, sides of the germband stage embryo; a, antenna; pr. ser, presumptive serosa; ser, serosa; t1, thoracic segment/leg 1. Micrographs in panels a, c, e are adapted from Panfilio et al. (2006), Fig. 3.
(in the case of the amnion). The previously separate amnion and serosa (re-)fuse over the embryo’s head, at the former site of invagination. With the two membranes firmly attached to each other, they rupture within the area of contact, freeing the head and antennae (Fig. 3c). As the serosa contracts toward the anterior pole of the egg, the internal amnion and embryo emerge and replace the serosa. The embryo occupies the ventral surface while the amnion provides a provisionally dorsal and lateral covering over the yolk. Emergence of embryo from yolk and membranes entails a 180° revolution, a backflip. As the embryo progressively emerges—first head and antennae, then the legs, and lastly the abdomen—it also bends backwards over the posterior pole to reach the ventral surface. Thus at mid katatrepsis, it forms a U-shape—half in, and half out of the yolk (Fig. 3d). By the completion of katatrepsis, all tissue has emerged from the yolk, and the embryo has advanced until the head is near the anterior pole of the egg (Fig. 3e). The serosa, formerly covering the entire egg surface, compacts into a small cap at the anterior pole.

Once katatrepsis is complete, the serosa is no longer required. It sinks behind the head, forming a structure called the (secondary) dorsal organ, and degenerates. Meanwhile, the flanks of the embryonic body grow dorsally-laterally and replace the amnion, creating a complete body cavity that occupies the full volume of the egg. Remaining yolk is now internal, and the embryonic gut forms around it. The amnion, made redundant by embryonic dorsal closure, probably degenerates as well (but see the ‘Future directions and questions’ section).

The axes of the egg are defined as corresponding to those of the embryo at hatching (and possibly also to the mother during oogenesis: Cobben, 1968; Wheeler, 1893), and are constant. Implicit in the above description, blastokinesis causes the embryo’s orientation to change with respect to the axes of the egg. Invagination at anatrepsis proceeds by a caudad-first entry of tissue into the yolk. The embryonic tissue remaining near the site of invagination, at the posterior pole, develops into the embryonic head. Further, the ventral side of the embryo faces upward: into the yolky interior and toward the dorsal side of the egg. Thus the germ-band stage embryo is both upside down and backward (note axis key in Fig. 3). This situation is corrected by the movements of katatrepsis, after which axes of egg and embryo correspond.

Notes on terminology

“Blastokinesis,” “anatrepsis,” and “katatrepsis” were coined by Wheeler (1893). The latter two derive from the Greek roots aná (“up”) and kata (“down”), referring to the direction of motion of the embryo as it passes in an arc over the posterior pole. “Blastokinesis” (Gr.: blastos, “bud, embryo”; kinesis, “movement”) encompasses both events, though usually with emphasis on the latter. Indeed, some authors have referred to “katatrepsis” as “blastokinesis” (Butt, 1949; Cobben, 1968; Dorn, 1976; Enslee and Riddiford, 1981; Johannsen and Butt, 1941; Miller, 1939; Slifer, 1932; Truckenbrodt, 1979; Woodland, 1957). In the older literature, katatrepsis is referred to as “revolution” (e.g., Kershaw, 1914; Knower, 1900; Melander, 1903; Seeger, 1979; Slifer, 1932). “Anatrepsis” is sometimes equated with, or considered part of, “gastrulation” in a broad sense (Kelly and Huebner, 1989; Roth, 2004), although it is distinct from the formation of the inner layer, or mesoderm, which may occur simultaneously with, after, or before anatrepsis (e.g., Goss, 1952; Heming, 1979; Krysan, 1976, respectively). The interval between anatrepsis and katatrepsis was originally referred to as “diapause” (Wheeler, 1893), but the terms “intertrepsis” (Heming, 2003; Heming and Huebner, 1994; Masumoto and Machida, 2006) or simply “germband stage” (e.g., Liu and Kaufman, 2004) are now more commonly used. “Blastokinesis” is also used to describe changes in embryo position or shape in the entognathans, the Archaeognatha, and the Lepidoptera, but these are recognized as distinct processes from blastokinesis in hemimetabolous insects (Fig. 2; denoted “Bx,” see Boxes 1 and 2; Anderson, 1972a; Jura, 1972; Wheeler, 1893). Here I use “blastokinesis” to mean the specific movements of anatrepsis and katatrepsis.

Morphogenetic events involving the extraembryonic membranes have been described by an array of terminology. In particular, there is a problem with the term “revolution,” which usually means katatrepsis but also has been applied to longitudinal rotations of the germband (described below in ‘Variations on katatrepsis, including rotations’; e.g., Rosay, 1959), or even changes in the curvature of the germband (Box 1; e.g., Kobayashi, 1998; Patten, 1884). Historically, insect embryo researchers tended to interpret data so that they fit contemporary terminology and paradigms, and now “blastokinesis” and “revolution” have come to mean any movement of the embryo within the egg. Here I apply these terms more strictly: “revolution” refers to the backflip movement at katatrepsis; “rotation” applies to movements about the longitudinal axis of the egg.

For the following comparative survey, types of blastokinesis and of extraembryonic membrane formation are indicated in Fig. 2 and summarized in Table 1.

Types of anatrepsis

“Anatrepsis” describes movements whereby the ventral-posteriorly positioned germ rudiment (= presumptive embryo + amnion) “moves through an arc till its body is completely inverted” in an “ascending movement” (Wheeler, 1893). In superficial anatrepsis, the mode that defined the term, the arc of movement is dictated by the posterior pole, which the embryo slides over in reaching the dorsal surface of the egg. Alternatively, in immersion anatrepsis, the ascending movement is synonymous with invagination of the germ rudiment into the yolk, as in Oncopeltus, preempting movement fully onto the dorsal surface. Superficial and immersion modes reflect fundamental differences in the relationship of anatrepsis to membrane formation (discussed in the following subsection). Furthermore, the limited available evidence suggests that these modes occur by distinct processes (discussed below in ‘Why does blastokinesis occur?’). Although superficial and immersed anatrepsis represent discrete categories, the actual manifestation of anatrepsis in different species is impressively complicated.
Table 1
Morphogenetic variations in insect extraembryonic development

<table>
<thead>
<tr>
<th>Degree of blastokinesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full blastokinesis: anatrepsis + katatrepsis (A/K)</td>
</tr>
<tr>
<td>Katatrepsis without anatrepsis (I_k)</td>
</tr>
<tr>
<td>Non-blastokinetic (N)</td>
</tr>
</tbody>
</table>

Method of extraembryonic membrane formation

- by extension/overgrowth from folds at the periphery of the embryonic rudiment (black triangle, ▲)
- by extension/overgrowth of membrane tissue from free leading edges (white triangle, △)
- by production of a sac-like pocket via invagination (black circle, ●)

Germband positional type

- Immersed (“I” in katatreptic orders, “i” in non-katatreptic orders)
- Superficial (“S” in katatreptic orders, “s” in non-katatreptic orders)

Other movements known as “blastokinesis”

- Embryonic postural change from ventrally convex to concave (B1; see Box 1)
- ‘Proto-blastokinetic’ involving a pro-amnion and a pro-serosa (B2; see Box 1)
- Lepidopteran blastokinesis (B3; see Box 2)

Symbols refer to the notation used in Fig. 2.

Superficial anatrepsis and persistent superficial growth (Fig. 2: “A”, “I”, and “S”) occurs in some Phasmida (stick insects; Bedford, 1970). Superficial anatrepsis followed by sinking to an immersed position in the yolk (Fig. 2: “A”, “I”, and black triangles—symbols described below and in Table 1) is seen in: some Blattaria (cockroaches: Heymons and Heymons, 1905; Lenoir-Rousseaux and Lender, 1970), the Grylloblattodea (rock crawlers: Uchifune and Machida, 2005), other Phasmida (cited in Bedford, 1970), and some Orthoptera (crickets and grasshoppers: Rakshpal, 1962). The orthopteran Gryllus assimilis has a particularly elaborate manifestation of superficial movement: the germ rudiment is superficial and first posterior, then dorsal, then ventral before finally sinking into the yolk from the ventral surface and ending up immersed near the dorsal side of the egg (Rakshpal, 1962).

Immersion anatrepsis (Fig. 2: “A”, “I”, and black circles) is exhibited by species of the basal pterygote order Odonata (dragonflies and damselflies: Ando, 1955; Seidel, 1929), and by nearly all of the Paraneoptera (bugs, thrips, lice: Butt, 1949; Cobben, 1968; Goss, 1952; Heming, 1979; Mellanby, 1935; Miura et al., 2003; Oseto and Helms, 1972; Sander, 1959; Seeger, 1979; Seidel, 1924). The site of invagination into the yolk is generally at or near the posterior pole (Fig. 4a), although a few species exceptionally invaginate from the ventral surface (e.g., some Hemiptera: Cobben, 1968; Psococoea [lice]: Seeger, 1979). The degree of immersion within the yolk following invagination varies, even within a single suborder (e.g., the heteropteran Hemiptera: Cobben, 1968), with some species positioned deep within the yolk, while others are only covered by a thin film of yolk on either the ventral or presumptive dorsal (e.g., Oncopeltus: Fig. 3b) side of the embryo. Partial immersion in the yolk occurs in some Odonata, where the embryo is immersed “for only half its length” (cited in Cobben, 1968, p. 310; and Jura, 1972, p. 91). Aside from relative position within the yolk, degree of immersion—after both types of anatrepsis—includes whether or not attachment to the serosa is maintained. Often a thin film of yolk may intervene between the serosa and internal tissue, or the tissues may be separate but apposed (Cobben, 1968; Johannsen and Butt, 1941). Persistent attachment to the serosa has been described within the Odonata, for some Orthoptera, and some Hemiptera (Cobben, 1968, p.96, 308).

A curious hybrid of immersed and superficial anatrepsis is seen in both suborders of the Orthoptera (Caelifera: Bentley et al., 1979; Ensifera: Heymons, 1895; Sarashina et al., 2005; Vollmar, 1972; Wheeler, 1893). The germ band is essentially superficial, but during later anatrepsis, the cephalic region of the developing germ band does not follow the caudal region over the surface of the posterior pole in reaching the dorsal surface. Rather, this portion of the embryo “takes a short cut through the yolk system” (Vollmar, 1972), such that it is temporarily immersed.

There is variation in the extent to which full blastokinesis occurs. In nearly all hemimetabolous species, the germ band stage embryo is positioned with its head remote from the anterior pole of the egg, upside down and backward with respect to the egg’s axes. However, in species where the germ rudiment is small and originates at or near the posterior pole, an inverted position is attained without any posterior–dorsalward movement in an ascending arc, and therefore these species lack anatrepsis but have later katatrepsis (Fig. 2: “/k”). This condition is seen in species regardless of whether they are superficial or immersed, and include: the Thysanura (firebrats: Heymons and Heymons, 1905; Masumoto and Machida, 2004; Masumoto and Machida, 2006; Woodland, 1957), Ephemeroptera (mayflies: Tojo and Machida, 1997), Isoperta (termites: Hu and Xu, 2005; Knower, 1900), Embiddina (web-spinners: Kershaw, 1914; Melander, 1903), Plecoptera (stoneflies: Kishimoto and Ando, 1985; Miller, 1939, 1940), and Dermaptera (earwigs: Bhatnagar and Singh, 1965; Heymons, 1895). Similar to development involving true anatrepsis, early development in some of these taxa exhibits variation in topographical details such as: a ventral site of invagination (some Plecoptera), partial immersion in the yolk (some Thysanura, the Isoptera), persistent attachment to the serosa (some Plecoptera), and even persistently open amniotic cavities (the Thysanura: references as above and Hughes et al., 2004).

Lastly, in the recently described—and still controversial—new order Mantophasmatodea (heel-walkers or gladiators: Cameron et al., 2006; Klass et al., 2002), the sole account of embryology to date covers only later stages of embryogenesis (Machida et al., 2004). In species of this order, embryos attain an immersed position and likely undergo katatrepsis, but it is an outstanding question as to whether there is anatrepsis and what type it may be (Fig. 2: “/k”).

Some insects lack anatrepsis and katatrepsis, or are non-blastokinetic (Fig. 2: “N”). A few notable exceptions within the hemimetabolous insects that follow this pattern are: ovoviparous Blattaria (Bullièere, 1969; Wheeler, 1889), Mantodea (praying mantids, known only from: Hagan, 1917), and Coranus species of Hemiptera (Cobben, 1968, pp. 299–300). In contrast, within the Holometabola, non-blastokinetic growth is predominant in all described orders (Anderson, 1972b):

Extraembryonic membrane formation and anatrepsis

Species differ in the nature of extraembryonic membrane formation and the extent to which it is related to anatrepsis. If the germ rudiment invaginates into the yolk as a sac (Fig. 2: black circle, Figs. 3a, 4a), the act of invagination defines extraembryonic membrane formation: sac formation separates the germ rudiment from the serosa and delimits the amniotic cavity. Thus in species with immersion anatrepsis, anatrepsis causes membrane formation, as in the Odonata (Seidel, 1929) and Paraneoptera (Goss, 1952; Heming, 1979; Johannsen and Butt, 1941; Mellanby, 1935; Miura et al., 2003; Oseto and Helms, 1972; Seeger, 1979; Seidel, 1924). (However, invagination does not predicate anatrepsis if there is no inversion of embryonic orientation, which is the case in: Ephemeroptera (Tojo and Machida, 1997), Plecoptera (Kishimoto and Ando, 1985; Miller, 1939, 1940), exceptional species of Coleoptera (Krysan, 1976) and Trichoptera (Miyakawa, 1974), and basal Lepidoptera (Ando and Tanaka, 1980; Kobayashi and Ando, 1981).

In other species the amnion differentiates earlier: prior to or during movement in anatreptic species, and usually prior to germ band elongation in non-blastokinetic species. In the differentiated blastoderm, the periphery of the germ rudiment is the presumptive amniotic tissue (Figs. 5a–5j). This peripheral tissue then roofs over the embryo proper in a characteristic fashion in many taxa (Fig. 5a, Fig. 2: denoted by black triangles; described in: Anderson, 1972a; Roth, 2004). Amniotic tissue posterior to the embryo, and then also lateral and anterior to each of the head lobes, arises as folds that migrate medially until the tissue meets and fuses, creating an intact amniotic cavity overlying the embryo. As the amniotic tissue migrates, it remains attached to the rest of the extraembryonic blastoderm, which gets dragged with it. This outer tissue then fuses with itself medially and disengages from the amnion, thus creating the intact serosa. Variations on the folding pattern just described include the order of formation of the folds and the number of folds that develop (e.g., Beeman and Norris, 1977; Patten, 1884; Roomal, 1936). The driving force for fold outgrowth is unclear, and may differ between species, such as by serosal proliferation and extension over the germ rudiment (Thomas, 1936), or by the germ rudiment pushing below the presumptive extraembryonic tissue (Woodland, 1957). Outgrowth of folds is the predominant mechanism of membrane formation across the Insecta, including the Holometabola, and occurs in: Thysanura (Hughes et al., 2004; Masumoto and Machida, 2006; Woodland, 1957), Isoptera (Knowler, 1900; Truckenbrodt, 1979), Blattaria (Bullière, 1969; Lenoir-Rousseaux and Lender, 1970; Wheeler, 1889), Mantodea (Hagan, 1917), Embiidiina (Kershaw, 1914; Melander, 1903), Dermaptera (Heymons, 1895), Grylloblattodea (Uchiyune and Machida, 2005), Phasmdida (Bedford, 1970; Thomas, 1936), Orthoptera (Bentley et al., 1979; Dearden et al., 2000; Rakshpal, 1962; Roomal, 1936; Wheeler, 1893), Coleoptera (Handel et al., 2000; Kobayashi et al., 2002; Rempel and Church, 1969; Stanley and Grundmann, 1970), Neuroptera (Bock, 1941; Du Bois, 1938; Miyakawa, 1979; Strindberg, 1915), some lower Hymenoptera (Ivanova-Kasas, 1959), Trichoptera (Kobayashi and Ando, 1990; Patten, 1884), Siphonaptera (fleas: cited in Anderson, 1972a, p. 202; and Miyakawa, 1975), Mecoptera (scorpionflies, cited from two unpublished sources in Miyakawa, 1975), and some lower Diptera (Idris, 1960; Raminani and Crippa, 1975, 1978).

Within the Holometabola, a third mechanism of membrane formation is seen in some Lepidoptera (Anderson and Wood, 1968; Gross and Howland, 1940; Kobayashi, 1998; Nagy et al., 1994; Okada, 1960) and some Hymenoptera (e.g., the honeybee: Bull, 1982; Fleig and Sander, 1988; Lamer and Dorn, 2001; Pultz et al., 2005; Shafig, 1954). Here the intact serosa is formed
before the germ rudiment differentiates into amnion and embryo. This uncoupling of membrane formation is achieved because of a break in continuity of the blastoderm tissue. The serosa and germ rudiment both acquire free leading edges (instead of being joined at folds or at the boundary of an invagination) that migrate over underlying tissue independently (Fig. 2: denoted by white triangles). In the honeybee this even leads to serosal and (vestigial) amnion formation by tissue migration in opposite directions—ventrally and dorsally, respectively. In the higher Lepidoptera, a typical amnion forms from a single fold, but after the detached germ rudiment has sunk into the yolk (Kobayashi, 1998). Some hymenopteran species show even greater divergence in membrane development, with very late serosal membrane formation and no amnion (e.g., Bull, 1982; Shafiq, 1954). Holometabolous species with a reduced extraembryonic membrane complement (‘Phylogeny’ section, above) accordingly lack (complete) amnion formation.

In summary, there are temporal and topographic differences between extraembryonic membrane morphogenesis via invagination, from marginal folds, or from free edges. However, the invaginating and folding modes of amnion formation are similar in that the posterior region of the amniotic membrane is typically formed first. Indeed, the final covering of the head lobes by anterior amniotic folds can occur very late in development in invaginated embryos (Butt, 1949; Seidel, 1929).

**Germband stage (intertrepsis) positional types**

It is during the germband stage that the embryonic events often studied by developmental geneticists occur, including overt segmentation, early appendage formation, and early neurogenesis. As part of segment formation or patterning, the germband elongates. Some embryos even temporarily become longer than twice the egg length (e.g., plataspid hemipterans: Cobben, 1968, p. 121). Within the confines of the egg such extension involves bending of the embryo. Thus many embryos—immersed or superficial—have characteristic flexures along their length. Often the caudal region is flexed such that the posterior abdomen curls back toward the head of the embryo on its ventral side (Fig. 4b). Depending on the position of the head region, embryos will form variously a J-shape (Seidel, 1929), a C-shape (Cobben, 1968), or an S-shape (Seeger, 1979). Many variations on these shapes occur, including ‘reverse’ shapes due to dorsal abdominal flexure (Fig. 5b). The degree of immersion in the yolk also differs between species at this stage.

**Variations on katatrepsis, including rotations**

Katatrepsis generally occurs halfway through development and is rapid, often comprising less than 1% of total developmental time (Anderson, 1972a; Cobben, 1968). This phase of blastokinesis reverses the inverted position of the embryo resulting from anatrepsis (or from embryonic development from the posterior pole of the egg, Fig. 2: ‘‘/K’’). Immersed embryos exit through the original site of invagination, superficial embryos slide back down over the posterior pole, and some orthopterans repeat their short cut through the yolk. Exceptionally, species that became immersed from a longitudinal position take a new route and also exit the yolk at the posterior pole (Cobben, 1968; Rakshpal, 1962; Wheeler, 1893). Once repositioning of the embryo is sufficiently complete, growth of the embryonic flanks over the yolk closes the embryonic body dorsally, and subsequent development (organogenesis) proceeds. If the embryo is still small relative to the egg at this stage, such as in the Orthoptera, the correctly oriented embryo may still be remote from the anterior egg pole, in which case dorsal closure also involves anteriorward growth of the body (e.g., Bentley et al., 1979).
Various changes to the extraembryonic membranes are associated with katatrepsis. Events in Oncopeltus are typical of many species: at the posterior pole the amnion and the serosa fuse over the embryo's head (Fig. 4d), and then the membranes rupture (Fig. 4e); the serosa contracts, leaving the embryo and evertent amnion in its stead on the egg surface (Fig. 4f, g); the serosa condenses into a small cap (dorsal organ) and degenerates (Fig. 4h); the amnion serves as a temporary covering for the yolk and is then replaced by the embryo during dorsal closure. In non-blastokinetic species, the amnion and the serosa still generally fuse with one another, rupture, and then contract anteriorly/dorsally, though the site of initial membrane rupture is at the ventral midline of the egg and below the anterior/thoracic region of the embryo (Fig. 5c, d; e.g., Bullière, 1969; Hagan, 1917; van der Zee et al., 2005; Wheeler, 1889). Subsequent dorsal closure is the one event in these proceedings that occurs in Drosophila, and here the dynamics of molecular and mechanical interactions between the amnio-serosa and the leading edge epidermal cells are largely understood (Edwards et al., 1997; Harden, 2002; Hutson et al., 2003; Jacinto et al., 2000, 2002; Kiehart et al., 2000; Köppen et al., 2006; Laplante and Nilson, 2006; Reed et al., 2001; Stronach and Perrimon, 2001; Wada et al., 2002; Young et al., 1993). In contrast, which tissue drives katatrepsis has not been experimentally investigated and may differ between species (cf. Enslee and Riddiford, 1981; Slifer, 1932), offering a ready subject for biomechanical, morphodynamic research across the insects.

The roles of the amnion and the serosa can be uncoupled from one another to varying extents during late extraembryonic movements, particularly within the Holometabola. These variations concern the occurrence of membrane rupture, the degree of coordination of amnion and serosa, and how provisional dorsal closure over the yolk is attained. For example:

- In the planthopper Siphanta acuta (Hemiptera), besides the amnion and serosa, there are two indusial envelopes (additional membrane covers peculiar to some species). The inner of these participates with the amnion in membrane rupture and contraction at katatrepsis, leaving the surrounding serosa and outer indusial membrane intact until hatching (described in Johannsen and Butt, 1941). On the other hand, a single indusial envelope may aid the serosa during membrane contraction in the mantid Paratenodera sinensis, and it subsequently forms a second dorsal organ (Hagan, 1917).

- In apocritan Hymenoptera with an amnionic vestige, amniotic tissue develops so as to cover the yolk rather than the embryo from the beginning. Dorsal closure occurs within the intact serosa, and the amnion is merely replaced without any attendant membrane rupture (Fleig and Sander, 1988).

- Similarly, in the cyclorrhaphous Diptera (e.g., Drosophila), the vestigial amnio-serosa serves as a provisional dorsal cover throughout its existence, and is also merely replaced during dorsal closure (Campos-Ortega and Hartenstein, 1997).

- In the (nearly) amnionless Hymenoptera, dorsal closure is effected without the amnion and within an intact serosa (Ivanova-Kasas, 1959; Shafiq, 1954).

- In some Coleoptera and Lepidoptera, dorsal closure occurs within an intact serosa and amnion, with dramatic changes in the latter. As in other species, at the germband stage the amnion covers the ventral surface of the embryo, and is joined to the embryo at its lateral flanks. A second part of the amnion then grows dorsally from the embryonic flank region to create the provisional dorsal covering of the yolk below the serosa. Definitive, embryonic dorsal closure ensues. The two parts to the amnion persist until hatching in this configuration (cited in Anderson, 1972b; Beeman and Norris, 1977; Kobayashi, 1998).

The ultimate fate of the extraembryonic membranes differs between species. Usually the amnion and the serosa degenerate. However, in some species either one or both of the membranes persist and are merely shed at the time of hatching (Anderson, 1972b; Ivanova-Kasas, 1959; Shafiq, 1954; Wheeler, 1893). Exceptionally, irrespective of blastokinetic type (Box 2), some lepidopteran prolarvae ingest amnion, serosa, and yolk prior to hatching (Anderson and Wood, 1968; Kobayashi, 1998; Okada, 1960; Reed and Day, 1966), though species in the most basal lineage exhibit typical dorsal organ formation and subsequent membrane degradation (Kobayashi and Ando, 1981).

A final variation on katatrepsis concerns the orientation attained by the embryo at the completion of movements. In Oncopeltus katatrepsis restores the orientation of the embryo to that of its original position at the germ rudiment stage and to that of the egg. However only the anterior–posterior, but not the dorsal–ventral, axis is restored in some taxa, due to longitudinal rotation of the embryo. Rotations may occur at any stage during embryogenesis, and also in non-blastokinetic species: during anatrepsis (uniquely within the phasminids: Bedford, 1970, 1978; Moscona, 1950), throughout germband stages (Abbassy et al., 1995; Hagan, 1917; Rakshpal, 1962; Rosay, 1959), at katatrepsis, at later developmental stages (Bentley et al., 1979), or in some combination of these time points (for an extended review of rotation, see: Cobben, 1968, pp. 298–300 and 307–308). Rotation specifically around the time of katatrepsis may be the most common, and occurs in Odonata, Isoptera, some Orthoptera, Thysanoptera (thrips), and some Hemiptera (Anderson, 1972a; Cobben, 1968; Heming, 1979; Hu and Xu, 2005; Sander, 1959; Truckenbrodt, 1979). A single 180° rotation of the embryo just prior to katatrepsis is typical. When rotation is not through a full 180°, there are sometimes two instances of rotation at different developmental stages such that the sum total is still a 180° about-face. However, departures from 180° rotation range from only 90° through “irregular prolarval [late developmental stage] rotations … through several circles” (Cobben, 1968, p. 300). When rotation is simultaneous with katatrepsis, it occurs as a “half-turn corkscrew roll,” and may be due to asymmetric fusion of the amnion to the contracting serosa (Cobben, 1968, p. 308), or to periodic, asymmetric contractions of the embryonic flanks (Truckenbrodt, 1979).

Overall, there are many variations on blastokinesis in the hemimetabolous insects, and indeed on extraembryonic membrane morphogenesis across the Insecta. However, modes of movement are generally conserved among related species. As
noted by Cobben (1968, pp. 310, 380), “within the Order or Suborder the embryonic shape is almost constant ... Family groups are distinguished by different types of embryogenesis.” Mapping blastokineti c and membrane formation traits onto a phylogeny of the Insecta reveals a great deal of variation within the lineage (Fig. 2), implying evolutionary plasticity. The diversity of the details contrasts with the conservation of blastokinesis as a general phenomenon that arose—probably only once—in the insects and was subsequently lost multiple times (in some Blattaria and some Hemiptera, in the Mantodea, and in the lineage leading to the Holometabola). Any phylogenetic signal that may be detected in these trends is certainly limited or noisy. Nevertheless, the ‘proto’ events in the Archaeognatha (Box 1), transitional features in the Thysanura (‘Phylogeny’ section, above), and then the appearance of full blastokinesis in the Pterygota, are suggestive of a conserved evolutionary trajectory of elaboration and (predominantly) conserved retention of the phenomenon.

**Relationship of the extraembryonic membranes to blastokinesis, and growth of the embryo**

The connection between extraembryonic membrane ontogeny and blastokinesis has long been recognized: “The elimination of the envelopes is preceded by katatrepsis just as their formation was preceded or accompanied by anatrepsis” (Wheeler, 1893). This coordination may represent an evolutionarily conserved feature, given that it is manifest throughout the hemimetabolous Pterygota (excepting only the Mantodea, based on a sole account: Hagan, 1917). It is also such that the two phenomena can be confused as defining one another. In accounts of holometabolous development, researchers have discussed manifestations of “blastokinesis” in their non-blastokineti c species, when in fact they are variously referring to membrane formation and degeneration, germband longitudinal rotation, or germband extension and retraction (Abbassy et al., 1995; Ivanova-Kasas, 1959; Rempel and Church, 1971; Stanley and Grundmann, 1970).

Germband extension has sometimes been analogized to, or mistaken for, anatrepsis. However, changes due to increased length are distinct from the anatrep tic movement (cf. Heming, 2003; Ivanova-Kasas, 1959; Stanley and Grundmann, 1970). In full anatrepsis, the head moves posteriorly and dorsally until it resides at the posterior pole or even on the posterior dorsal surface. This is not the case in species that lengthen over the posterior pole of the egg without an associated shift of the head (like *Drosophila* and the Isoptera: Campos-Ortega and Hartenstein, 1997; Hagan, 1917; Knower, 1900), or even with an anterior shift over the anterior pole of the egg (Fig. 5b; such as the wasp Nasonia: Bull, 1982; and Tribolium beetles: Stanley and Grundmann, 1970; van der Zee et al., 2005).

A more complicated relationship of membranes and movements pertains to germband retraction in some holometabolous species. Here rupture of the extraembryonic membranes is coordinated with embryo retraction, and retraction also returns the posterior region of the embryo to the ventral side (many Coleoptera, also Neuropteroidea, Trichoptera, and Mecoptera: Anderson, 1972b). Even in the derived condition in *Drosophila*, the reduced amnioserosa is required for proper germband retraction, as evidenced from mutants affecting this tissue and from mechanical studies (Frank and Rushlow, 1996; Hamburger et al., 2004; Irish and Gelbart, 1987; Lamba and Lipshtiz, 1999; Reim et al., 2003; Schöck and Perrimon, 2002; Wakimoto et al., 1984). Heming (2003) sees the membranes’ role in proper germband retraction as a manifestation of blastokinesis. However, this is not katatrepsis. Hemimetabolous embryos also undergo germband extension and retraction (Figs. 3b, c, 4b, c, 5b, c), but retraction is unrelated to katatrepsis, as the two events are temporally distinct. Often germband retraction occurs prior to katatrepsis (Butt, 1949; Hu and Xu, 2005; Mellanby, 1936; Uchifune and Machida, 2005), but it can also occur afterward (Miura et al., 2003). Perhaps even more convincingly, within the holometabolous Coleoptera, some species also exhibit germband retraction before non-blastokinetic membrane rupture (Beeman and Norris, 1977; Rempel and Church, 1971). Thus, in most species germband retraction is not physically dependent on membrane topography. The dependence of some holometabolous embryos on the extraembryonic tissue for proper retraction represents a consequence of a novel topographical relationship, rather than a subtle version of katatrepsis.

In sum, the Holometabola are, with the derived exception of some Lepidoptera (Box 2), non-blastokinetic, although they derive from insects that have blastokinesis. Confusion is understandable given the frequent co-occurrence of membrane and embryo ontogenetic events and the lack of suitable terms to distinguish them. In particular, extraembryonic membrane rupture/contraction is the morphogenetic event that occurs either at the initiation of katatrepsis or prior to non-blastokinetic dorsal closure (except in taxa with reduced extraembryonic membranes). However, as an event that flows seamlessly either into katatrepsis or dorsal closure, it has been linguistically subsumed under either “katatrepsis” or “dorsal closure,” even by researchers working within the same taxa (Rempel and Church, 1971; van der Zee et al., 2005). Which term is used reflects whether the researcher’s background is in the classical, comparative tradition or in the realm of *Drosophila* developmental genetics. Similarly, it is probable that some insect endocrinologists refer to hemimetabolous katatrepsis (Kidokoro et al., 2006) or holometabolous late membrane development (Palma et al., 1993) as “blastokinesis” because early work in the field was conducted on lepidopterans (Riddiford and Williams, 1967). Although not catchy, “membrane rupture/contraction” is certainly more precise if katatrepsis or lepidopteran blastokinesis is not intended.

So how do membranes and movements relate? Anatrepsis can contribute to differentiation of amnion, serosa, and embryo, but membrane formation and anatrepsis can also be independent. Immersion in the yolk may result directly from anatrepsis or by simple sinking. In later development, the occurrence and timing of membrane rupture/contraction and dorsal closure can also vary extensively between taxa, though the lack of blastokinesis in the Holometabola may contribute permissively to the extent of this variation. The only causal relationships are (i) if anatrepsis...
Non-insect hexapods and species of the earliest-branching insect lineage (see phylogeny in Fig. 2) undergo movements that reposition the embryo and involve extraembryonic tissue. These events are known as blastokinesis, though the entognathan event ("B1") is analogous to insect blastokinesis while the archaeognathan event ("B2") may represent an evolutionary precursor.

In entognathan "B1" blastokinesis (Fig. 6a), the ventrally convex, superficial germband ducks into the yolk, resulting in a ventrally concave posture as the embryo doubles over on itself (Ikeda and Machida, 1998, 2001; Jura, 1967, 1972; Machida, 2006; Uemiya and Ando, 1987). The primary dorsal organ, an entognathan-specific structure distinct from the dorsal organ of hemimetabolous insects (Fig. 4h), is required for B1: if it is ablated, the germband persists in the ventrally convex position and cannot hatch (Table 2; Jura, 1967). The role of the primary dorsal organ in effecting B1 is unclear, although it may alter the yolk such that the embryo can push into it (Jura, 1967).

A B1-like postural change occurs in some holometabolous insects (Anderson and Lawson-Kerr, 1977; Kobayashi, 1998; Kobayashi and Ando, 1981, 1990; Kobayashi et al., 2002; Patten, 1884; Storch and Krysan, 1980), and even in some crustacean embryos (Browne et al., 2005). The authors of these studies variously referred to it as "revolution," "a blastokinetic movement," or simply as "ventral flexure." This trait contains no phylogenetic signal and probably only represents initially convex growth over the yolk surface followed by later folding of the developed embryo within a restrictive egg space. Across the Holometabola there is no correlation of the timing of this folding with either extraembryonic membrane rupture/contraction or dorsal closure.

In Archaeognatha, the early egg surface consists of the embryonic rudiment, a pro-amnion, and a pro-serosa; the latter two are distinguished by differing nuclear densities (Jura, 1972; Machida et al., 1994). Archaeognathan "B2" blastokinesis (Fig. 6b) involves a folding movement that tucks the early embryo into the yolk at a large crease in the egg surface (Heymons and Heymons, 1905; Jura, 1972; Larink, 1997; Machida, 1981; Machida et al., 1994). The tissue on the same wall of the crease as the embryo is the pro-amnion while the tissue on the opposing wall may be the pro-serosa (Heymons and Heymons, 1905) or more of the pro-amnion (Machida et al., 1994). B2 differs from membrane formation in other insects, as the pro-amniotic and pro-serosal regions are relatively static while shape changes result from yolk folds. Also, B2 does not create a discrete amniotic cavity, since the space within the crease is continuous with the external space. Interspecific differences in B2 concern the stage of embryonic development when it occurs, the number of yolk folds produced, and the extent to which the pro-serosa is involved. This variation is in marked contrast to the coordination of extraembryonic membrane formation with anatrepsis in other insects ("Relationship of the extraembryonic membranes to blastokinesis" section), and may represent an evolutionary stage before these events became so tightly coupled and precise in execution.

Fig. 6. Other movements known as blastokinesis, illustrated by schematic cartoons of topographical changes of the embryo (grey), amnion (orange), serosa (light blue), and primary dorsal organ (dark blue), for (a) "B1" in collembolans and (b) "B2" in archaeognathans, after Uemiya and Ando (1987) (and my unpublished observations) and Heymons and Heymons (1905), respectively. Images are in the lateral aspect with egg-anterior up and egg-dorsal right. The embryo’s head is the enlarged region in dark grey. To convey the relative size and ventral side of older embryos, dark grey lines denote the three leg pairs and the antennae and light grey space-filling areas are used in some image panels. Black arrows indicate the direction of motion, including the reverse of ‘pro-anatrepsis’ in the final panel for B2.
occurs, katatrepsis must also occur (see the following two sections), (ii) katatrepsis may occur without anatrepsis if the germband is inverted with respect to the egg anyway, and (iii) at the time of dorsal closure the embryonic body must be (come) roughly one egg length.

**Why does blastokinesis occur?**

Although anatrepsis and katatrepsis are usually classed as morphogenetic movements, the general consensus—as true now as it was over 100 years ago—is that “nobody knows what these movements are good for” (Sander, 1976). The Holometabola demonstrate that blastokinetic movements and even the extraembryonic membranes can become dispensable over evolutionary time, and throughout the Insecta the extraembryonic tissue is a temporary attribute of the egg (but see the ‘Future directions and questions’ section). Nonetheless, given how striking and taxonomically widespread blastokinetic or membrane overgrowth + uncovering are, invariably questions of adaptive, functional, or mechanical significance arise.

There are numerous speculations on possible functions of blastokinesis. These usually concern anatrepsis, as katatrepsis is less problematic. There is a yin and yang aesthetic to anatrepsis followed by katatrepsis, with a sense of balance implicit in the literature. What anatrepsis does, katatrepsis then undo. Katatrepsis has been described as “an active restorative movement” (Anderson, 1972a), “when the more mature germband retraces the route it took earlier” (Sander, 1976). Thus, insofar as the germband stage position resulting from anatrepsis is untenable for future development, katatrepsis can easily be explained. One consideration, already mentioned, is that incorporation of yolk into the developing gut requires a position external to it. More generally, the germband stage embryo consists largely of external, ectodermal structures. A surface position then creates space into which organs and other internal structures can grow during the second half of embryogenesis. Anderson (1972a) also points out that in many species the ventral surface of the egg is convex, whereas the dorsal surface is concave, such that coincidence of egg and embryo ventral surfaces “most economically” accommodates the elongation of the appendages. Anterior–posterior orientation may also matter: the cuticular “egg burster” employed by some embryos during hatching develops on the head, and in some species it is most effectively applied to a structurally weaker area of the chorion, which is usually at the anterior end of the egg (Cobb, 1968). Thus katatrepsis may be seen as an “accommodatory movement” (Anderson, 1972a, p. 131).

Why, then, does anatrepsis occur in the first place? Some arguments for the occurrence of anatrepsis are based on physiological considerations. Uniquely, Wheeler (1893) suggested that blastokinesis comprises the movements of the developing embryo away from localized regions of yolk pollution from metabolism (anatrepsis), and later aeration of the yolk bodies when churned up by the older, larger embryo (katatrepsis). However, regulation of metabolic wastes is now attributed to the amniotic cavity (‘Proposed functions of the extraembryonic membranes’ section, above). Alternatively, some authors have suggested that immersion (via anatrepsis) may facilitate yolk “mobilization” by the germband stage embryo, perhaps by increasing the surface area of embryo in contact with the yolk (Heming, 2003; Miura et al., 2003). However, in both the superficial and immersed situations, the presumptive dorsal side of the embryo is in contact with the yolk and the amnion serves as an intervening cover on its ventral side. Thus it is not apparent that immersion increases the surface area of embryonic tissue in direct contact with its nutrient source, and these arguments do not account for superficial anatrepsis.

**How does it occur?: Insights from experimental data**

As is often the case, when the “why” questions are presently intractable, the “how” questions are a good way to proceed. Happily for research on extraembryonic specification and morphogenesis, steps have already been taken in this direction (Table 2). In the pre-developmental genetics era of experimental embryology, various means of poking and prodding the embryos identified some relevant experimental tools and uncovered possible components to extraembryonic morphogenesis. Often the experimental phenotypes generated by these manipulations were variable. In the last few years, the possibilities for selective, genetic manipulation by RNA interference (and transgenesis) have been—and continue to be—extended to an increasing number of species representing several orders, including the Orthoptera, Hemiptera, Coleoptera, Hymenoptera, Lepidoptera, and Diptera (e.g., Amdam et al., 2003; Brown et al., 1999; Bucher et al., 2002; Dong and Friedrich, 2005; Huang et al., 2007; Hughes and Kaufman, 2000; Konet et al., 2007; Liu and Kaufman, 2004; Lynch and Desplan, 2006; Meyering-Vos et al., 2006; Mito et al., 2005; Miyawaki et al., 2004; Sanchez-Vargas et al., 2004). Phenotypic data from RNAi have already identified several genes relevant to extraembryonic developmental processes in two species. These genetic data, coupled with awareness of other relevant manipulative techniques and with candidate gene information from Drosophila studies, provide an ample foundation for future research.

**Early extraembryonic development**

At present, little is known about the molecules involved in extraembryonic membrane specification. Outside of extensive studies on genes necessary for Drosophila amnioserosa formation and differentiation (e.g., Hamaguchi et al., 2004; Irish and Gelbart, 1987; Reim et al., 2003; Rusch and Levine, 1997; Wakimoto et al., 1984), the only functional data to date come from RNAi studies on the zen1 gene, which encodes a homeodomain transcription factor, in the beetle Tribolium castaneum. After knockdown of Tc-zen1, the presumptive serosal tissue becomes transformed to a more ventral/posterior fate as embryonic tissue (van der Zee et al., 2005). This is similar to the phenotype obtained in Drosophila zen mutants (Wakimoto et al., 1984).
Data on the impetus for anatrepsis come primarily from physical manipulation studies, and suggest that it may be, in part, a byproduct of various physical forces acting in the early egg. A number of early studies (described in Heming, 2003; and in Sander, 1976), such as Sander’s (1959, 1960, 1967) work on anatrepsis in the leafhopper *Euscelis plebejus* (Hemiptera), implicate the yolk system (consisting of various types of yolk granules as well as cytoplasmic net and nuclei) in effecting anatrepsis. The germ rudiment is passively pulled into the yolk at the posterior pole as a consequence of concentric, anteriorly directed contractions of the yolk system, these contractions being associated with cleavage of the yolk in some species. The

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**Box 2**

Innovations in the Holometabola: Monster cells and more

Holometabolous insects are non-blastokinetic but exhibit an array of innovations in extraembryonic development. Membrane reduction and varied deployment during rupture/contraction are discussed in the main text (‘Proposed functions’ and ‘Variations on katatreps’ sections). A few additional intriguing examples are mentioned here.

Apoditrysian Lepidoptera (higher moths, butterflies) have a unique movement known as blastokinesis, here designated B₃ (Fig. 7; Anderson and Wood, 1968; Okada, 1960; Reed and Day, 1966; Riddiford and Williams, 1967). The immersed germband completes dorsal closure within the amnion, and has a ventrally convex flexure. The embryo then sucks the amniotic fluid into the body from the mouth, expanding the body’s volume and producing a ventrally concave flexure. This postural change is reminiscent of that achieved in entognathan blastokinesis (B₁, Box 1), but by an entirely different process. Similar to hemimetabolous katatrepsis, this type of blastokinesis is perturbed by treatment with ectopic juvenile hormone (Table 2; Erezyilmaz et al., 2004; Riddiford and Williams, 1967).

Although typically the serosa degenerates or is simply shed at hatching, parasitoid Hymenoptera (wasps) have found a postembryonic use for serosal cells. The eggs and larvae of these species develop inside a host animal, an environment that poses certain challenges. After the embryonic stage, the serosa dissociates into individual cells—termed teratocytes (literally “monster cells”)—that circulate in the host’s hemocoel (Danyk and Mackauer, 1996; Pedata et al., 2003; Pennacchio et al., 1994; Rouleux-Bonnin et al., 1999). Functions of the teratocytes may include sequestration of the host’s: nutrients (aids parasitoid feeding/causes host attrition), hormones (altering host development in a manner favorable to the parasitoid), or immune factors (suppressing a response to the parasitoid) (Dahlman, 1990).

Within the Diptera (flies), some species have both amnion and serosa, while the Cyclorrhapha, including *Drosophila*, have the single amnioserosa (Schmidt-Ott, 2000). Recent findings on extraembryonic membrane specification in the (non-cyclorrhaphan) mosquito Anopheles gambiae (Goltsev et al., 2007) imply that changes occurred in the developmental process itself, enabling such a reduction. In *Anopheles*, some genes are expressed in either the amnion or the adjacent serosa. The *Drosophila* orthologues of these genes are expressed uniformly throughout the amnioserosa. The authors suggest that the distinction of amnion and serosa became lost in the Cyclorrhapha when these genes became expressed in overlapping domains (Goltsev et al., 2007). In most insects, tissue territories become distinguished in the order [serosa, germ rudiment [amnion, embryo]] (Roth, 2004). The *Anopheles* data imply that this sequence has changed within the flies, with the specification sequence [embryo, extraembryonic [amnion, serosa]]. Restructuring of genetic regulation, such that the serosa and amnion derive from a common field of cells, would represent a profound change in the developmental process itself. If this is the case, the dipteran amnion may be unrelated to the amnion of most insects.

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Fig. 7. “B₃” blastokinesis in higher Lepidoptera, illustrated by schematic cartoons of topographical changes of the embryo (grey), amnion (orange), and serosa (blue), after Anderson and Wood (1968). Images are in lateral aspect with egg-anterior up and egg-dorsal right. The embryo’s head is the enlarged region in dark grey. To convey the relative size and ventral side of the embryo, dark grey lines denote the three leg pairs and the antennae and light grey space-filling areas are used. Black arrows indicate the direction of motion, including ingestion of egg contents by the embryo in the third panel (the portion of the amnion over the mouth has already been consumed in this image).
Table 2
Summary of functional perturbations of extraembryonic morphogenesis (in species other than *Drosophila melanogaster*), with manipulations listed in order of the developmental timing of the affected event

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Affected event</th>
<th>Phenotype</th>
<th>Order: Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Affecting extraembryonic tissue formation</strong></td>
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<tr>
<td><em>Tc-zen1</em> RNAi</td>
<td>Serosa differentiation</td>
<td>Tissue transformation to embryonic fate: no serosa, temporarily enlarged head</td>
<td>Coleoptera: Tribolium castaneum</td>
<td>van der Zee et al., 2005</td>
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<tr>
<td><strong>Affecting early extraembryonic morphogenesis</strong></td>
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<tr>
<td>Encasement in glass (axonia? pressure?)</td>
<td>Blastoderm differentiation; anatrepis</td>
<td>Failed blastoderm differentiation and incomplete invagination</td>
<td>Hemiptera: <em>Gerris paludum insularis</em></td>
<td>Mori, 1985</td>
</tr>
<tr>
<td><em>Of-dpp</em> RNAi</td>
<td>Anatrepis/germ rudiment differentiation</td>
<td>Failure of germ rudiment invagination or morphological differentiation</td>
<td>Hemiptera: <em>Oncopeltus fasciatus</em></td>
<td>Angelini and Kaufman, 2005</td>
</tr>
<tr>
<td>Transverse cutting of the embryo</td>
<td>Anatrepis</td>
<td>Continued migration of the posterior portion only; anterior portion no longer moves</td>
<td>Orthoptera: <em>Acheta domesticus</em></td>
<td>Vollmar, 1972</td>
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<tr>
<td><strong>Affecting late extraembryonic morphogenesis</strong></td>
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<td><strong>Gene-targeted treatments:</strong></td>
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<tr>
<td><em>Tc-zen2</em> RNAi</td>
<td>Membrane rupture/contraction</td>
<td>Complete or partial eversion</td>
<td>Coleoptera: Tribolium castaneum</td>
<td>van der Zee et al., 2005</td>
</tr>
<tr>
<td><em>Of-hb</em> RNAi (phenotypic class V)</td>
<td>Katatrepsis</td>
<td>Complete eversion</td>
<td>Hemiptera: <em>Oncopeltus fasciatus</em></td>
<td>Panfilio et al., 2006</td>
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<tr>
<td>Chemical and hormonal treatments:</td>
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<tr>
<td>Actinomycin D</td>
<td>Katatrepsis</td>
<td>Complete or partial eversion; anterior–posterior inversion, inability to hatch; death at an earlier developmental stage</td>
<td>Isoperta: <em>Odontotermes badius</em></td>
<td>Truckenbrodt, 1979</td>
</tr>
<tr>
<td>Various juvenile hormone (JH) mimics</td>
<td>Katatrepsis; dorsal closure; lepidopteran blastokinesis (B3)</td>
<td>Failed or prematurely arrested movements: small body size, aberrant body shape, open body walls and unincorporated yolk</td>
<td>Orthoptera: <em>Acheta domesticus</em>, <em>Locusta migratoria</em>, <em>Schistocerca gregaria</em></td>
<td>Erzyilmaz et al., 2004; Kidokoro et al., 2006; Novák, 1969; Riddiford and Williams, 1967</td>
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<tr>
<td><strong>Other treatments:</strong></td>
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<tr>
<td>Ligation (constriction)</td>
<td>Katatrepsis</td>
<td>Complete or partial eversion</td>
<td>Odonata: <em>Calopteryx atrata</em>, <em>Cercion hieroglyphicum</em></td>
<td>Ando, 1955; Mori, 1975; Sander, 1959, 1960</td>
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<tr>
<td>Cauterization (heat ablation)</td>
<td>Katatrepsis; entognathan blastokinesis (B1)</td>
<td>Complete or partial eversion; failure of movement</td>
<td>Hemiptera: <em>Gerris paludum insularis</em>, <em>Euscelis plebejus</em></td>
<td>Mori, 1975; Jura, 1967</td>
</tr>
<tr>
<td>Cold treatment</td>
<td>Katatrepsis</td>
<td>Anterior–posterior inversion; inability to hatch</td>
<td>Hemiptera: <em>Gerris paludum insularis</em></td>
<td>Neuhaus, 1967</td>
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<td></td>
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<td></td>
<td>Coleoptera: <em>Tetrodontophora bielanensis</em></td>
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<td></td>
<td></td>
<td></td>
<td>Phasmida: <em>Bacillus llibanicae</em></td>
<td>Moscona, 1950</td>
</tr>
</tbody>
</table>

Note that a “complete eversion” phenotype also implies anterior–posterior inversion in katatreptic species.

The main site of activity has been described as an “anatrepis center,” and visible foci fitting this description have been seen and/or experimentally demonstrated in hemipterans (reviewed in Heming and Huebner, 1994). Of course, this is an explanation for the immersion type of anatrepis only. However, the yolk system is also thought to contribute to the dorsal shift occurring in superficial anatrepis, via peristaltic contractions, in conjunction with the crawling action of filopodia on the posterior of the germ rudiment (Vollmar, 1972).

A recent contribution to the body of experimental data on anatrepis comes from maternal RNAi of the signaling molecule *decapentaplegic* (*dpp*) in the bug *Oncopeltus* (Angelini and Kaufman, 2005). Depletion of *Of-dpp* in this manner results in a failure of germ rudiment invagination (=immersion anatrepis) or differentiation: cells of the germ rudiment form a round, external mass at the posterior pole of the egg. The cells have some degree of embryonic identity, as in older eggs this tissue acquires pigmentation typical of the embryo (Panfilio et al., 2006), but there is no apparent morphological differentiation. The cells are also capable of initial morphogenetic movements, as posteriorward migration and condensation occur in a fashion similar to wild type, but the tissue does not then enter the yolk. The serosa appears unaffected. The site of the *Of-dpp* RNAi defect correlates with the site of *Of-dpp* expression at the posterior pole (Angelini and Kaufman, 2005). Thus the TGF-beta/Dpp signaling pathway is implicated in this morphogenetic event, although the exact role of *dpp* in invagination remains unknown. This role for Dpp signaling is distinct from its role in *Drosophila* in dorsal tissue specification, which includes specification of the amnioserosa (Irish and Gelbart, 1987; Rusch and Levine, 1997).
Late extraembryonic development

Most functional data to date for extraembryonic development pertain to late morphogenetic events, especially katatrepsis or non-blastokinetic membrane rupture/contraction (Table 2). As most of the manipulations employed in these studies were open-ended regarding which developmental stage would be affected, these results do not reflect a popular interest in katatrepsis or the non-blastokinetic equivalent a priori. Rather, the fact that multiple manipulations specifically perturb this event suggests that it is a particularly sensitive stage. For example, in one species, half of all spontaneously occurring embryonic defects involve defective katatrepsis (Panfilio et al., 2006). There is also anecdotal evidence that environmental stress may impair katatrepsis (Ando, 1955). These observations correlate with the fact that the katatrepsis or membrane rupture/contraction stage is a period of high activity and change within the egg, including de novo transcription (Truckenbrodt, 1979) and synthesis of new classes of proteins (Handley et al., 1998).

The most commonly observed phenotype for perturbed katatrepsis or membrane rupture/contraction is evasion of the embryo. That is, the embryo completes development in an inside-out fashion, with the organ tissue outside and the appendages inside of the body wall. Such a striking phenotype results from a failure of extraembryonic membrane rupture/contraction, which leaves the embryo in its germband position (Fig. 1), surrounded ventrally by the amnion. At the time of body flank outgrowth for what ought to be dorsal closure, the embryonic lateral flanks grow in a direction dictated by the inner surface of the amnion, such that the appendages are enclosed ventrally.

Everted insect embryos have been produced experimentally since the 1950s, and recent RNAi data contribute to this body of work. Various treatments have induced evasion in species representing several insect orders: by ligation in dragonflies (Odonata); by actinomycin D inhibition of transcription in termites (Isoptera); by treatment with ectopic juvenile hormone mimics in locusts (Orthoptera); by ligation, zen2RNAi, or hunchbackRNAi in bugs (Hemiptera); and by zen2RNAi in the red flour beetle (Coleoptera) (Ando, 1955; Erezyilmaz et al., 2004; Kidokoro et al., 2006; Mori, 1975; Novák, 1969; Panfilio et al., 2006; Sander, 1959, 1960; Truckenbrodt, 1979; van der Zee et al., 2005).

In sum, mechanical manipulation of the egg, inhibition of transcription (of all genes), inhibition of translation (of targeted genes), or inappropriate hormone levels can lead to evasion. Thus although dramatic, the phenotype of evasion is not uncommon.

Eversion can be complete or partial. Complete eversion may be due to a failure of initiation of membrane contraction/rupture events (Panfilio et al., 2006; van der Zee et al., 2005). Alternatively, complete eversion may result from a delay that misses the correct developmental time window, resulting in late serosal contraction that leaves the amnion and embryo behind (Ando, 1955; Truckenbrodt, 1979). In either situation, the proximate cause of membrane rupture has yet to be determined. The latter situation highlights the timing of membrane rupture/contraction—or of contextual factors—as an important focus for future research.

Partial eversion may result from several different defects. If amnion-serosa fusion is incomplete or if amniotic membrane integrity is compromised, the amnion may tear during serosal contraction and leave the embryo partially covered by membrane. The position of tearing then corresponds with the demarcation between uneverted and everted portions of the embryo (Truckenbrodt, 1979). Partial eversion could also result from inappropriate timing of dorsal closure with respect to katatrepsis, for example, arrest in the U-shaped, mid katatrepsis position (Fig. 3d), leaving the posterior portion of the embryo in the amniotic cavity to become everted (e.g., Novák, 1969). In ligatured dragonfly eggs, interference with the normal pressures in the egg may have caused cessation of contraction during katatrepsis (Ando, 1955). On the other hand, in Oncopeltus, hunchbackRNAi-mediated partial eversion results from a molecular cause and likely has a specific effect on different portions of the egg and/or embryo (Liu and Kaufman, 2004).

Partial eversion due to arrest at the mid katatrepsis position occurs after orthopteran embryos are treated with juvenile hormone (JH) mimics (Erezyilmaz et al., 2004; Kidokoro et al., 2005; Novák, 1969). However, this phenotype is just one of the diverse array of defects that ectopic JH activity can produce, depending on the particular substance used, concentration, and timing of treatment. For example, in the cricket Acheta domesticus, early JH treatment (egg age 0–5 days) can lead to arrest at mid katatrepsis (on day 7), but treatment during the 2 days prior to katatrepsis does not have an effect (Erezyilmaz et al., 2004), suggesting that hormone levels indirectly affect katatrepsis. Similarly, the effects were seen several days after JH treatment in Locusta migratoria, where treatment suppressed the completion of katatrepsis while other developmental events proceeded (Kidokoro et al., 2006). One of the JH mimics up-regulated MAPK/ERK signaling (Kidokoro et al., 2006), although it is unclear whether this signaling pathway is relevant for katatrepsis or other events. In general, hormone levels represent an additional factor that affects extraembryonic morphogenetic events, though hormonal changes are unlikely to be involved on the short-term time scale in the initiation of events.

Overall, successful membrane rupture and subsequent repositioning of membranes and embryo are crucial for normal development, and these developmental events are sensitive to perturbation. It has been recognized for some time that, “study of everted embryos might produce very useful information about the mechanism of katatrepsis, one of the most characteristic features of insect embryogenesis” (Mori, 1975). Induced eversion has even been employed as a means of studying the development of usually internal anatomical structures (Mori, 1977). In practical application, widespread susceptibility to eversion makes extraembryonic membrane rupture/contraction an attractive target for insect pest control via specific, molecular strategies (Wimmer, 2005). Conservation of zen2RNAi-induced eversion across representatives of the Hemiptera and Coleoptera—species-rich orders containing many agricultural and some pathogenic pests—immediately suggests one possible target.
RNAi of specific genes—which causes consistent developmental failure at a particular step—offers the least complicated approach for future studies, as the technique produces one or only a few phenotypes and avoids possible confounding factors for egg health that physical manipulations cause. The toolkit will expand as additional genes are characterized, building a molecular understanding of the basis of these morphogenetic events.

**Future directions and questions**

Insect extraembryonic development is a wide-open field that is tractable for future research. Presently, we have individual data points for physical, molecular, and endocrine factors involved in some processes, but linking these points into a larger framework is still to come. There are also some outstanding questions from twentieth century work, as well as new considerations in light of recent studies.

As enigmatic as ever are the developmental origin and final fate of the amnion. Which cells on the surface of the blastoderm contribute to the amnion? The location and number of domains comprising the germ rudiment differ between species, and remain unclear in others (e.g., Butt, 1949; Mellonby, 1935; Sarashina et al., 2005; Seidel, 1924), and this has implications for where presumptive amniotic cells are located. Alternatively, in some species, the amnion may not be prefigured in the blastoderm but rather arise *de novo* during invagination or fold formation (Machida et al., 1994; Tojo and Machida, 1997; Woodland, 1957). These hypotheses are based on classical observation and histology. There is a substantial need for the definition of molecular markers for the amnion and, more generally, for fate mapping and lineage tracing of early stages in diverse insects.

It is not merely a polite euphemism to say that the amnion is “replaced” by the embryonic flanks during dorsal closure (e.g., ‘Variations on katatrepsis’ section). In fact, there are no data to provide a more explicit account of the tissue’s fate. During dorsal closure, does it contract toward the midline to aid closure (Dorn, 1976; Woodland, 1957) like the *Drosophila* amnioserosa (Kiehart et al., 2000), migrate anteriorly to join the serosa in the dorsal organ (Kershaw, 1914; Patten, 1884), remain *in situ*? Subsequently, where does the amnion go—degenerate into the yolk (Machida et al., 1994) or contribute to the embryonic epidermis (Truckenbrodt, 1979; Woodland, 1957)? The latter question especially has troubled researchers throughout the past century (e.g., Jura, 1972, p. 90). Again, suggested possibilities are based on inspection of fixed specimens. In *Drosophila* it is only recently that fluorescent live imaging analyses conclusively showed that the amnioserosa degenerates after dorsal closure (Reed et al., 2004; contra Techau, 1987).

Between blastokinetic events, the posited functions of the membranes when they are static and cover the developing germ band embryo suggest avenues for further, experimental work on aspects of physiology (e.g., cuticle production, water regulation) and innate immunity (‘Proposed functions’ section, above).

The *Tribolium zen2* and *Onycopeltus zen* RNAi data show that this transcription factor is involved in the initiation of katatrepsis or membrane rupture/contraction. What are its molecular targets, and for what purpose (cytoskeletal regulation and cell shape changes, changes in cell polarity)? More generally, what is the trigger for membrane rupture? As rupture is a rapid process that occurs within minutes (Cobben, 1968, p. 33), perhaps it is precipitated by a physiological signal such as intracellular calcium release, which is involved in vertebrate morphogenesis (Slusarski and Pelegri, 2007). Equally, the proximate triggers for membrane outgrowth as folds, invagination for immersion anatrepsis, or migration for superficial anatrepsis are largely unknown.

Insect extraembryonic morphogenesis provides subject matter for research on diverse epithelial morphogenetic processes: folding, invagination, dissociation to create free edges, fusion, rupture, contraction, and reorganization during degeneration. As cells are constrained by their positions within the epithelium and have defined cell neighbors, the diversity of movements achieved by the amnion and the serosa are all the more impressive. As many morphogenetic events involving the membranes occur on the egg surface and within a few hours, they are accessible and ideal for live imaging, a technique that is already regularly used in *Drosophila* dorsal closure studies. Fundamental aspects of cell shape changes, establishing polarity for directed movements, and intercellular communication at tissue boundaries have yet to be explored. Parameters of a mechanical or physical nature are virtually unknown and await characterization for these movements. The identification of some transcription factors and extracellular signaling molecules necessary for these morphogenetic events provides a promising starting point.

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**References**


