Supplementary Figures:

Supplementary Figure 1. Changes in growth zone dimensions plotted by developmental Stage

Data from panels A-C in Figure 1, replotted by developmental stage, both as averages and as box and whiskers. Data paired in Figure 1, e.g., germband length and area, are on separate graphs in this case for greater visibility. ANOVA of stages (performed using GraphPad Prism 6 software) support claims made in Figure 1 (e.g., comparisons among stages typically show no significant differences with adjacent stages but differences with non-adjacent stages, developmental stages taken from timed embryo cohorts and have n values ranging from 5 to 25). There is a significant trend in “last segment area”: there the data generally fall into two clusters, with the first 6 EN stage separated from the remaining ones. This demarcation is right at the thoracic/abdominal boundary when the rate of segmentation increases.
Supplementary Figure 2. Position dependent fate of all blastoderm clones co-mapped with temporal pattern of segment addition

*Tribolium* embryo with 14 of the 16 EN stripes counted in this study (EN 1-3 = Md, Mx, Lb; EN 4-6 = T1- T3; EN 7-16 = A1-10). On the left, under the label A., white lines indicate average number of segments added during the 2 h intervals used in this study. Between 16 and 18 h, less than a segment is added. Between 18-20 h over 5 segments are added. (The average number of segments added in each interval are as follows: 1.8, 2.8, 0.7, 5.2, 2.1). On the right, all the clone distributions of this study are mapped along the body length, with segment numbers under the label B. Original blastoderm position, as percent egg length, is above each group of mapped clones. Note that the 16-18hr time point, when only 1 segment is added, correlates with a position in the embryo that marks a transition between more anterior clones that contribute to 1-3 segments within the gnathal and thoracic segments and more posterior clones that contribute to 2-8 segments within the abdomen. Note that the anterior most clone in Fig.2 of the manuscript is not included in this diagram as it mapped anterior to the mandibular segment.
Supplementary Figure 3. Segment addition data analyzed as linear regression versus separate timepoints

Data from Figure 3 replotted as a linear regression (A) and shown in box and whiskers plot (B). A. Linear regression of number of segments against embryo age shows a high correlation ($R^2=0.89$, $n=148$) for a calculated segmentation rate of about 45 min per segment. This overall rate matches previously published rates calculated in the same fashion, i.e., by assuming linearity. However, this analysis hides the temporal variability in segment addition. B. Box and whiskers plot to show specimens with identical values to clarify measures with the same value not visible in A. Analysis performed using GraphPad Prism 6 software.
Supplementary Figure 4. Replicates of timing of segment addition.
Data presented for segment addition are the third replicate and are highly repeatable, thus excluding rearing conditions (e.g., fluctuations in the incubator) as an explanation for the variability in periodicity. Supplementary Table 1 shows all three replicates, numbers scored and dates. We used the final replicate since all measures indicated in Figure 1 E were taken on all specimens included in it. However, early replicates of just EN stripe measures versus hAEL are highly consistent with the submitted one.

Supplementary Table 1

<table>
<thead>
<tr>
<th>Date scored</th>
<th>12 hAEL</th>
<th>14 hAEL</th>
<th>16 hAEL</th>
<th>18 hAEL</th>
<th>20 hAEL</th>
<th>22 hAEL</th>
<th>24 hAEL</th>
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</thead>
<tbody>
<tr>
<td>2012</td>
<td>4.3 (7)</td>
<td>6.4 (11)</td>
<td>7.7 (3)</td>
<td>12.0 (17)</td>
<td>15.0 (14)</td>
<td>16.0 (7)</td>
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<tr>
<td>early 2013</td>
<td>3.5 (20)</td>
<td>6.2 (26)</td>
<td>7.3 (44)</td>
<td>11.7 (55)</td>
<td>14.6 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>late 2013</td>
<td>2.2 (24)</td>
<td>3.9 (31)</td>
<td>6.7 (20)</td>
<td>7.4 (20)</td>
<td>12.6 (18)</td>
<td>14.7 (32)</td>
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</tbody>
</table>
Supplementary Note 1

Supplementary Information on Modeling

The simulation represents space as a regular two-dimensional lattice of pixels. Each cell in the simulation is represented as a domain of pixels on the lattice that share a common cell index, \( \sigma \). Simulation pixels that are not occupied by cells are occupied by Medium. The state of the simulation—which pixels “belong” to which cells—evolves via a stochastic modified Metropolis algorithm consisting of a series of index-copy attempts. For each attempt, the algorithm randomly chooses a target pixel and an adjacent source pixel. If the two pixels belong to different cells (or one to a cell and one to Medium), the target cell will attempt to assume the same index as the source pixel—that is, it will attempt to become a part of the same cell as the source pixel. Whether the attempt is successful depends on the resulting change in the effective energy of the simulation lattice (described below); the probability that the attempt will be successful is given by the Boltzmann acceptance function:

\[
P(\sigma(i) \rightarrow \sigma(j)) = \begin{cases} 
\frac{1}{e^\frac{\Delta H}{T_m}} & : \Delta H \leq 0 \\
1 & : \Delta H > 0 
\end{cases}
\]

where \( \Delta H \) is the change in the effective energy and \( T_m \) is a global parameter indicating the degree of random fluctuation allowed in the simulation. During each Monte Carlo Step (MCS), the algorithm makes \( N \) index-copy attempts, where \( N \) is the number of pixels in the simulation lattice.

The effective energy of the simulation lattice that determines the evolution of the simulation does not represent the actual energy of the simulated system. Rather, it specifies factors that constrain cell properties and behaviors in the simulation. Cells in our simulation have intrinsic random motility, experience position-dependent movements and have characteristic adhesion to other cell types in the simulation. The effective energy of the lattice is given by

\[
H = \sum_{i,j \text{neighbors}} J(\sigma(i), \sigma(j)) (1 - \delta(\sigma(i), \sigma(j))) + \sum_{\sigma} \left[ \lambda_{\text{vol}}(\sigma)(V(\sigma) - V_\text{t}(\sigma))^2 + \lambda_{\text{surf}}(\sigma)(S(\sigma) - S_\text{t}(\sigma))^2 \right]
\]

The first sum is over all pairs of neighboring pixels, \( i \) and \( j \), and calculates the contact energy between all neighboring cells. \( J(\sigma(i), \sigma(j)) \) is the boundary energy per unit contact area for the cells \( \sigma(i) \) and \( \sigma(j) \). The delta function ensures that only cell-cell interfaces contribute to the energy. Changes that lead to cell-cell interfaces with low contact energy will be favored over those that lead to cell-cell interfaces with high contact energy.

The second sum, over all cells in the simulation, calculates the contribution of the volume and surface-area constraints to the effective energy. Deviations from a cell \( \sigma \)'s target volume (\( V_\text{t}(\sigma) \)) or surface area (\( S_\text{t}(\sigma) \)) will increase the effective energy. Thus, changes that increase deviations from a cell’s target volume and/or surface area will be less likely to occur. The parameters \( \lambda_{\text{vol}} \)
and $\lambda_{\text{surf}}$ set the “strength” of these constraints: higher values of these parameters will lead to smaller fluctuations around the target values.

Growth-zone cells in our simulation also experience an imposed external force leading to position-dependent directed movement; in addition to the change in the effective energy above, index copies affecting these cells results in the following change in effective energy:

$$\Delta E_{\text{ext pot}} = \vec{u} \cdot (\vec{c} - \vec{c}_0)$$

where $\lambda_{\text{ext pot}}$ sets the magnitude of the potential experience by the cell, $\vec{u}$ is the direction of the imposed directed motion and $\vec{c}$ is the vector pointing from the source pixel to the target pixel. The result of this effective energy-change is that index-copy attempts that would result in a cell moving in its “preferred” direction are favored.

**Simulation cell behaviors**

Cells in our simulation have intrinsic random motility, experience position-dependent movements and have characteristic adhesion to other cell types in the simulation. Growth-zone cells in the anterior region of the growth zone periodically differentiate into segment cells. Simulation cells do not grow or divide.

**Simulation Cell Types**

There are three cell types in our simulation: growth-zone cells, even segment cells and odd segment cells. All cell types in the simulation preferentially adhere to cells of the same type, and adhere more strongly to cells of different types than to Medium. Cell contact energy parameters are given in below. The larger the contact energy parameter, the larger the effective energy penalty for contact between cells of the two types; the smaller the contact energy parameter, the more favored the contact between cells of the two types.

**Simulation cell-cell contact energy parameters**

<table>
<thead>
<tr>
<th></th>
<th>Medium</th>
<th>Growth Zone</th>
<th>Odd Segment</th>
<th>Even Segment</th>
</tr>
</thead>
<tbody>
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<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Growth Zone</td>
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<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Odd Segment</td>
<td>5</td>
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<td></td>
</tr>
<tr>
<td>Even Segment</td>
<td></td>
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<td></td>
<td>5</td>
</tr>
</tbody>
</table>

**Simulation parameters affecting cell motility**

The parameters $\lambda_{\text{surf}}$ and $\lambda_{\text{vol}}$ impact the degree of fluctuation about the target surface area (boundary length in two dimensions) and volume (number of pixels belonging to a cell) for cells in the simulation. Because cells in the simulation move by extending their boundaries (this can be compared to biological cells extending portions of their membranes), the greater the degree of fluctuation is, the greater the motility of the cells will be. $\lambda_{\text{surf}} = \lambda_{\text{vol}} = 2.0$ for all cells in the
simulation; this corresponds to a moderate degree of membrane fluctuation, leading to moderate cell motility in the simulation.

We also imposed position-dependent movement on growth-zone cells in the simulation. Directed cell movements were chosen to emulate measured cell migrations in the early Tribolium embryo (10). Our model does not posit a mechanism for driving these cell movements, nor are we forwarding the hypothesis that these particular patterns of cell movements drive elongation in the Tribolium germ band during the modeled stages. The simulation is intended only to demonstrate that realistic germ-band elongation can possibly arise from cell movements alone in the absence of a highly proliferative posterior growth zone.

**Segmentation algorithm**
Periodically (every 1200 MCS), growth-zone cells with centers within 60 pixels (seven to eight typical cell diameters) of the y-position (AP position) of the posterior-most segment cell switch cell types to become segment cells. Segments are made up alternately of odd segment and even segment cells. High contact energy between cells of the two segment types maintains segment boundaries in the simulation. This choice was made for convenience; while adhesion differences between segment compartments is believed to be an important mechanism in maintaining segment boundaries in vertebrate segmentation, we are not hypothesizing such a mechanism in Tribolium at this time. This choice does not impact the conclusions drawn from the simulation.

**Initial conditions**
Simulation initial conditions were chosen to emulate 17-18 h Tribolium growth zones in length (approximately 30 cells) and number of cells (approximately 660 cells).