Accelerated cell divisions drive the outgrowth of the regenerating spinal cord in axolotls

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Figure 1. Spinal cord outgrowth time-course during regeneration. (A) Representative images of a regenerating spinal cord after tail amputation (individual time-lapse images are in Figure 1—figure supplement 1). The white dashed line marks the amputation plane. The arrowheads mark the tip of the regenerating spinal cord. Scale bar, 1 mm. (B) Spinal cord outgrowth time-course during the first eight days after amputation (n = 8 axolotls). DOI: 10.7554/eLife.20357.002
**Figure 1—figure supplement 1.** Images used for spinal cord outgrowth measurements in *Figure 1B*. Each row shows images from an axolotl; each column shows animals from one time point analyzed. Vertical and horizontal green lines mark the amputation plane and the spinal cord outgrowth, respectively. High-resolution images are in *Rost et al., 2016b*. Animal t3 is shown in the representative images of *Figure 1A*.

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Figure 2. Cellular mechanisms underlying spinal cord outgrowth. (A) Sketch of measurements taken to estimate the density and total number of neural stem cells (nuclei, black dots) in the axolotl spinal cord. The density of SOX2\(^+\) cells, \(\rho\), is the ratio of the number of SOX2\(^+\) cells per cross section (# stem cells) and the mean AP cell length, \(l_c\). The density of SOX2\(^+\) cells is the proportionality constant between the total number of stem cells in a zone along the spinal cord with zone length, \(L_{sc}\). (B, B') Number of SOX2\(^+\) cells per cross section along the AP axis of a selected uninjured (B) and a selected day 4-regenerating spinal cord (B'). Black line and gray region indicate the mean number of SOX2\(^+\) cells and the standard deviation, respectively. Plots for all individual axolotls in Figure 2—figure supplement 1. (C) Spatial mean of the number of SOX2\(^+\) cells per cross section of individual axolotls against time (black dots). Black line and gray region indicate the mean number of SOX2\(^+\) cells and the standard deviation of all animals, respectively. (D, D') Number of SOX2\(^+\)/PCNA\(^+\) cells per cross section (upper panel) and mitotic cells per section (lower panel) along the AP axis in a selected uninjured (D) and a selected day 4-regenerating spinal cord (D'). Black line and the gray region show the expected number and the 68% confidence belt for the best fit of the model with two spatial proliferation zones, respectively. Plots for all animals in Figure 2—figure supplement 3. (E) Possible cell states in the two spatial proliferation zones model used to analyze the spatial cell proliferation dataset (D, D'). \(p_{\text{in}}\), probability that a cell is in the quiescent state; \(p_{\text{m}}\), probability that a cell is in the mitotic state; \(\Delta\) growth fraction, \(\Delta\) mitotic index.

Figure 2 continued on next page
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proliferative, otherwise quiescent. $p_m$, probability that a proliferative cell undergoes mitosis at the time of analysis. (E') The model assumes two proliferation zones. The location of the border between zones is called switchpoint. (F–F') Results of model fitting for growth fraction ($F$) and mitotic index time-course ($F'$) in the anterior (orange diamonds) and posterior (green triangles) zone. Error bars indicate the 68% credibility interval. (F'') Black dots mark the switchpoint. Blue dashed line marks 800 μm anterior to the amputation plane. The dashed region marks the space outside of the embryo, the dotted region marks the unaffected part of the embryo. (G) Proliferation rate time-course in the high-proliferation zone. (H) Total number of SOX2+/PCNA− cells in the high-proliferation zone (mean ± linearly propagated 1-σ error). (I) Selected time-lapse images of clone (blue arrowhead) tracking during spinal cord regeneration. Dashed line marks the amputation plane. Scale bar, 200 μm (J) Tracking of 19 clones along the AP axis during regeneration. Clone trajectories are color coded by their initial position. (K) Clone velocities at different positions along the AP axis.

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Figure 2—figure supplement 1. Number of SOX2$^+$ cells per cross section along the AP axis for all 15 animals. Each row shows data from three animals at a given time point. Data from animals 0D_1 and 4D_3 are shown as representative data in Figure 2B and B', respectively. DOI: 10.7554/eLife.20357.005
Figure 2—figure supplement 2. Simulation of the spatial model of cell counts to analyze the spatiotemporal pattern of cell proliferation. (A) Simulations of a spatially homogeneous zone of proliferation for three animals. Population mean number of stem cells per cross section, \( N_{\text{pop}} = 7 \), inter-animal standard deviation for number of stem cells per cross section, \( \sigma_{\text{pop}} = 1 \), intra-animal standard deviation for number of stem cells per cross section, \( \sigma = 0.5 \), probability of a cell to be proliferative (expected growth fraction), \( p_p = 0.5 \), inter-animal standard deviation of \( p_p \), \( \sigma_{p_p} = 0.04 \), probability of a proliferative cell to be mitotic (expected mitotic index), \( p_m = 0.015 \), inter-animal standard deviation of \( p_m \), \( \sigma_m = 0.003 \). (B) Simulations of two adjacent spatially homogeneous zones of proliferation for three animals. Parameters for the anterior zone are the same as in (A). The probability of a cell to be proliferative and probability of a proliferative cell to be mitotic in the posterior zone are elevated to \( p_p = 0.8 \) and \( p_m = 0.1 \), respectively. The mean switchpoint location is 300 μm anterior to the amputation plane and the corresponding inter-animal standard deviation is 100 μm. As expected, there are more proliferative and mitotic cells in the posterior zone. Simulation results can statistically be compared with the cell counts we obtained from experimentally observed animals to infer growth fraction, mitotic index and switchpoint (Figure 2F–F”).

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Figure 2—figure supplement 3. Number of SOX2⁺/PCNA⁺ cells per cross section (upper panel) and mitotic cells per section (lower panel) along the AP axis for all 15 animals. Data from animals 0D_1 and 4D_3 are shown in Figure 2D and D', respectively. Each row shows data from three animals at a given time point.
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Figure 2—figure supplement 4. Posterior marginal distributions for the parameters of the spatial model of cell counts to analyze the spatiotemporal pattern of proliferation. Each row shows a different model parameter. Each column shows a different time point. Three animals per time point were used.
Figure 2—figure supplement 4 continued

used in the analysis. Vertical dashed lines show the limits of the 95% credibility interval. The distribution means and the 68% credibility intervals for the growth fraction, mitotic index and the switchpoint are shown in Figure 2F–’.

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Figure 2—figure supplement 5. Cell cycle length time-course calculated from the proliferation rate time-course shown in Figure 2G.
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Figure 3. Mechanistic model of spinal cord outgrowth. (A) Sketch of cellular mechanisms included in the model: cell proliferation, quiescent cell activation, and cell influx into the 800 μm high-proliferation zone. (B) Growth fraction time-course of the SOX2⁺ cell population in the high-proliferation zone as observed (black dots) and fitted by the model (gray shaded areas, from darker to lighter, 68%, 95% and 99.7% confidence intervals of the model prediction). (C) Spinal cord outgrowth during the first eight days of regeneration as observed (black dots, n = 8 axolotls) and predicted by the model (Equations 1 and 2) (green shaded areas, from darker to lighter, 68%, 95% and 99.7% confidence intervals). The model prediction is in agreement with the experimental data. (D–G) Prediction of spinal cord outgrowth for four model scenarios based on Equations 1 and 2 with selected mechanisms switched off (green shaded areas). Black dots show the same experimental data as in panel (C). (D) The acceleration of the cell cycle is switched off. Hence, the proliferation rate is fixed to the basal proliferation rate of uninjured animals. (E) Cell influx is switched off (v = 0). (F) Quiescent cell activation is switched off (k = 0). (G) Cell influx and quiescent cell activation are switched off (k = 0, v = 0). Corresponding predictions for growth fraction in Figure 3—figure supplement 1.

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Figure 3—figure supplement 1. Prediction of the growth fraction in the high-proliferation zone for four model scenarios with selected mechanisms switched off (green shaded areas). Black dots show the same experimental data as in Figure 3B. Scenarios in panels A-D correspond to the scenarios in Figure 3D–G, respectively. Switching off the acceleration of the cell cycle length and switching off the cell influx hardly have an effect on the growth fraction time course (A, B). As expected, switching off the activation of quiescent stem cells has a strong impact on growth fraction time-course (C, D). This is consistent with the fit of a non-zero activation rate $k$ to these data.

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Figure 3—figure supplement 2. Comparison of the spinal cord outgrowth prediction by our model with the measured outgrowth reported by Fei et al. (2014). (A) Outgrowth prediction of the full model (green, same as in Figure 3C) and rescaled outgrowth in the control condition from Fei et al., 2014 (black dot, n = 12 axolotls). (B) Outgrowth prediction of the model for the case that cell cycle acceleration is switched off (green, same as in Figure 3D) and rescaled, density-corrected outgrowth in a Sox2-knockout condition from Fei et al., 2014 (black dot, n = 24 axolotls).

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Figure 4. Conceptual model of spinal cord growth during regeneration. Only one row of stem cells is shown as circles and three cell clones are marked with different patterns (striped, black and dotted). In the uninjured spinal cord (Day 0), cells divide at a slow, basal proliferation rate (white background). From day 4 after amputation, cells speed up their cell cycle and the growth fraction increases, within a high-proliferation zone that initially extends 800 μm anterior to the amputation plane (green background). The density of neural stem cells along the spinal cord stays constant and spinal cord outgrowth is achieved by an increase in the total number of neural stem cells. Acceleration of the cell cycle in the high-proliferation zone is the major driver of this increase in cell numbers. Dividing cells might push cells posteriorly. The more posterior a cell is the more cells anterior to that cell divide and push the cell making it move faster. While an anterior clone (striped) hardly moves, clones in the center of the high proliferation zone (black) move faster. Clones that start at the amputation plane (dotted) stay at the tip of the regenerating spinal cord and move fastest.

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