

MORPHOLOGICAL IMAGE ANALYSIS DEMONSTRATES THAT DEPLETION OF CATENIN P0071 RESULTS IN ABNORMAL EMBRYONIC WOUND HEALING

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Proteins of the p120 catenin family are important modulators of dynamic cell-cell adhesion. In cell culture studies, p120 family member p0071 was reported to function in cell adhesion and cell cytokinesis. In the present study we used *Xenopus* as a model system to assess the functional role of p0071 in development. An objective was to determine the effect of p0071 in embryonic epidermal wound healing, which is believed to be mediated by a contractile bundled actomyosin purse string in the epidermal cells flanking the wounds. Living embryos mounted in agarose were wounded using the high energy laser beam of a Leica sp5 laser scanning microscope. Repetitive scanning with full laser power at multiple focal planes resulted in rapid cell membrane damage and cell death in the outer epidermal layer. We compared cellular movement patterns after wounding between control and p0071 depleted embryos that express β -actin-GFP, allowing visualization of actin string formation and of cell-cell contacts. ImageJ [1] was used to track triple cell contacts on maximum intensity projections of z-stacks collected after wound induction. XY pixel coordinates in the digital images were used to calculate the distance to the wound centre for each cell at each time point. Data analysis demonstrated that movement of cells further away from the wound was substantially reduced in p0071-depleted embryos compared to controls. In p0071-depleted embryos however, cells bordering the wound compensated by showing increased lateral movement and flattening, which we demonstrated by measuring apical cell surface area. To investigate the possible mechanism behind these observations, we assessed the activation of the RhoA GTPase that was previously shown to be activated after binding with p0071. This was done by microinjecting a GFP-reporter construct that is recruited to sites of RhoA activity. GFP images were captured before, during and after laser wound induction. After wounding, alternating stacks of images were collected both at high and at low magnification. In control embryos, wounding resulted in an instant recruitment of the RhoA reporter construct to the subapical cortex of the epidermal cells surrounding the wound, followed by spreading of the RhoA activation zones in a wave-like pattern, progressing up to ten cell-diameters from the wound. Within one minute, the signal had become undetectable. In p0071-depleted embryos, RhoA activation was limited to cells in the close vicinity of the wound. Together, these results show that p0071 is essential for efficient wound closure dynamics by contraction of an actomyosin ring and closure by a purse-string mechanism. Depletion of p0071 leads to modification of the actomyosin skeleton and impaired RhoA activation, resulting in a compensational closure mechanism of stretching and flattening of the cells closest to the wound. Therefore, we conclude that p0071 is necessary for normal wound healing in *Xenopus*.

[1] W Rasband, ImageJ, U.S.National Institutes of Health, <http://rsb.info.nih.gov/ij/>, 1997-2009