Supporting Online Material for

The Embryonic Vertebrate Heart Tube Is a Dynamic Suction Pump

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This PDF file includes:

Materials and Methods
Figs. S1 and S2

Other Supporting Online Material for this manuscript includes the following:
available at www.sciencemag.org/cgi/content/full/312/5774/751/DC1

Movies S1 to S4

Methods

Embryo Preparation

Standard methods were used in spawning adult zebrafish and raising their embryos. Embryos were anesthetized in 0.0175% tricaine. 26 hpf Tg(*gata1*::GFP) embryos were placed ventral side up in wells etched into an agarose filled Petri dish. The orientation of the embryos was adjusted manually through the use of a small capillary tube.

Imaging

Embryos were raised at 28.5ºC and, unless otherwise noted, imaged at 24ºC. Bi-directional confocal scans (256x256 pixels) were taken at 151 frames per second (fps). Time series were triggered at a random time in the cardiac cylce and taken for 300-500 frames. Upon completion of a two-dimensional time series at one z-section, the optical plane was moved 3-5 microns and the acquisition was repeated. Four-dimensional datasets were collected from 15-25 z-sections and did not exceed 100 microns in total depth.

Quantitative Flow Analysis

Blood cell velocities were computed from image sequences, 5-10 cardiac cycles in length, acquired in fish at temperatures between 24ºC and 34°C. For every sequence, we selected the same portion of the heart near the center-line of the tube at the venous boundary as a region of interest. We manually marked the first frame of each cardiac cycle in which compression occurs defining cycles of variable length, or equivalently,

frequency. For every cardiac cycle, individual blood cell trajectories were tracked manually and pixel positions recorded over time. Cells were chosen that maintained their intensity values (indicating that their motion was largely in the focal plane) while traversing the region of interest. The instantaneous velocity of a cell was computed using the distance traveled between two frames along the angular orientation of the heart tube. For each cycle length, the velocities from sequences of corresponding duration were merged to yield a minimum of one velocity and up to twelve velocity measurements for each time step (Suppl. Fig.2). For all data corresponding to one cycle length ($\pm$ 0.007 seconds), a Monte Carlo sampling was performed. In this method, a velocity from each time step in a cycle is chosen at random. The average velocity for the given cycle length is then computed from these points. The random sampling is repeated 1000 times for each cycle length from which a mean and standard deviation of average velocities was computed (Fig. 3D). We tested the sensitivity of our results to the number of velocity points at each phase by computing average velocities only from phases that had at least three data values. Even under these stringent conditions, the non-linear frequency-flow relationship is conserved. The anticipated peristaltic rate was determined by assuming the heart was cylindrical and estimating the length of the actively compressing component during each cardiac cycle. We determined the average flow velocity by dividing the flow rate, which is equal to the volume compressed multiplied by the compression frequency, by the cross-sectional area.

Pressure Variation Estimates

In order to estimate pressure variations over time at a given position z along the heart tube axis, we utilized two known relations, (i) the oscillating pressure gradient with respect to time, dP/dt, is proportional to the spatial derivative, $\delta P/\delta z$, when δz is small (*11*), and (ii) the pressure is proportional to the inverse of the radius of the tube ($P= \alpha/R$, Laplace Law). Combining these two equations, we get

$$dP/dt \propto (R_1-R_2)/(R_1 R_2)$$

where R_1 and R_2 are the radii of the heart tube at neighboring cross sections. By measuring the latter radii over time (see Movie 4), and assuming a circular cross section, changes in pressure can be determined at the imaging time-resolution (±0.007 s).

Image Processing

Confocal images were collected and analyzed using the Zeiss 510 LSM software. Time series of two-dimensional sections were temporally registered in Matlab using a correlation process after conditioning data using a multiresolution wavelet transform (*8*). Four-dimensional data sets were analyzed using Imaris (Bitplane AG).