Longitudinal study of early Xenopus embryonic development using 3D-microscopic MRI

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Introduction: *Xenopus laevis* is a main model organism in the field of developmental biology. In the past, experimental embryology has defined many developmental concepts like axis formation or mesoderm- and neural induction. Since the advent of molecular biology, much has been learned about the molecular mechanisms of these concepts. In order to integrate this knowledge in the context of the whole embryo, tissue interrelationships in which the molecular mechanisms operate must be properly understood. However, due to the complete opacity of the early embryo at optical wavelengths, observations about the morphogenesis of the embryo remain incomplete and visualizations have relied on either fixed and sectioned material or on tissue explants. We aim to directly examine the development of the live frog embryo using microscopic magnetic resonance imaging (µMRI), and thus provide the bridge between both above-mentioned approaches. The initial feasibility of observing the development of the early frog embryo has been demonstrated (Jacobs and Fraser, Science 263 (5147), 1994). Here we explore the method in greater detail for the live examination of early Xenopus development in a true 3D time-lapse analysis.

Methods: Data acquisition was done using a 11.7T Bruker Avance MR spectrometer with microimaging capabilities. Images were acquired using a 3D-Spin-echo sequence (TR/TE=400 ms / 8ms, isotropic voxel size 39 µm) and a 2D-gradient-echo sequence (TR/TE=400 ms / 5ms, in-plane pixel size 23 µm, 200 µm slice thickness).

Results: Figure 1: comparison of water-proton (upper row) and fat-proton (lower row) with conventional optical images (middle row). Several embryonic regions can be distinguished by intrinsic contrast: the vegetal cell mass (VCM), the animal cap tissue (AC), the blastocoel (BC) and the archenteron (AR). The 39 µm voxel size and 55 min imaging time yield sufficient spatial and temporal resolution to visualize detailed changes in the morphology over time.

Figure 2: By injecting a T1-contrast agent (P717 Guerbet, France) into early blastomeres, we are able to follow the development of the labeled clone cells within the context of the whole embryo through early development in a longitudinal study. Coinjection of a fluorophore (TexasRed, Molecular Probes) allows the subsequent examination of the labeled cells after fixation by optical microscopy.

Figure 3: 2D-high resolution (23 µm pixel size) and high speed (6'40"imaging time) longitudinal image series of an early blastula stage Xenopus embryo. The nuclei of the early blastomeres can be identified and followed over several cell cleavages.

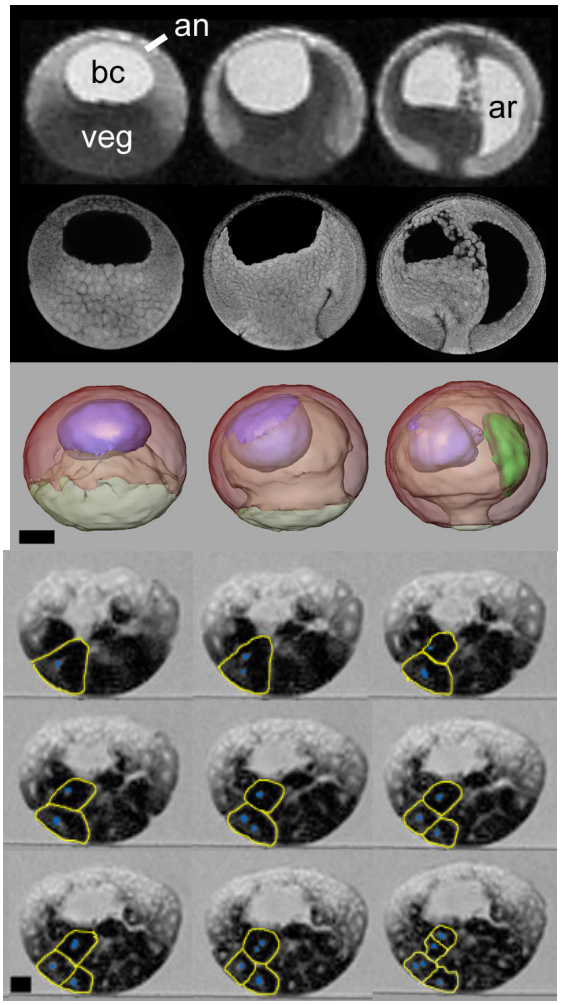

Figure 1: Upper row shows sagittal slices of 3D-image volumes. The major embryonic regions can be identified: veg = vegetal cell mass, bc = blastocoel, an = animal cap, ar = archenteron. Middle row shows sibling embryos fixed at the same time the MR images in the upper row were taken. Lower row shows surface renderings of the 3D-image data to give a better understanding for the three-dimensionality of the embryo. Scale bar: 300 µm.

Figure 3: High resolution 2D-images allow cell divisions of the early blastomeres of the *Xenopus* embryo to be followed in semi-real time. One example cell is highlighted in yellow, the nuclei in blue. Only every second scan is shown. Scale bar: 200 µm.

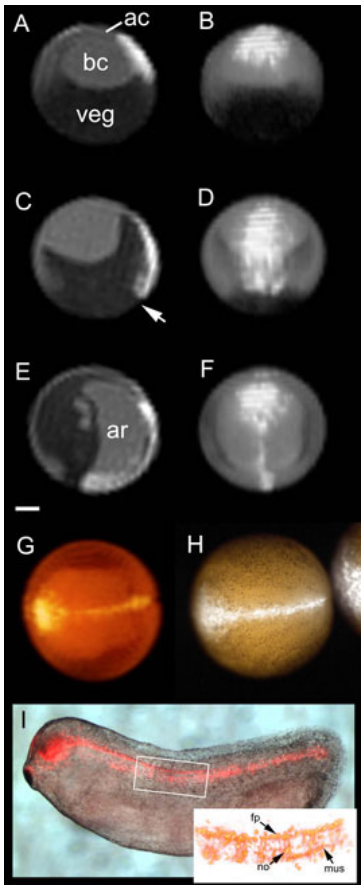

Figure 2. One blastomere was labeled at the 32-cell stage with P717 and TexasRed. The labeled descendants of the blastomere can be followed longitudinally thorough blastula and gastrula stages. A,C,E: lateral view, dorsal to the right. B,D,F dorsal view.

G shows a false colored dorsal view of the last scan of the time series, anterior to the left.

H Fluorescence microscope image of the same embryo immediately after the last MRI scan allows comparison of the labeled cells in both imaging modalities.

I: confocal microscope image of the same embryo at stage 28 after fixation and clearing. The labeled cells have differentiated into notochord (no) muscle (mus), floor plate (fp) and ventral nervous system. Scale bar: 200 µm.