- 3P340 形態形成と遺伝子発現をつなぐ細胞のインターカレーション
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- 3aK09 A cellular dynamics links gene expression to morphogenesis in the planar cell intercalation
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There are many reports that morphogenesis is correlated with gene expression. However, the cellular dynamics that links gene expression to morphogenesis is poorly understood. We have modified our 3D cell model of polyhedra (H.H. et al. *J. Theor. Biol.* **226**: 439-453, 2004) to describe that polyhedral cells change their shape under mechanical forces in a cell aggregate and to investigate the planar cell intercalation that leads to polarized morphogenesis.

During development, a tissue often elongates along one axis and narrows in the perpendicular axis without external force, where cells themselves intercalate actively among neighboring cells. The cell intercalation takes place in sea urchin gut elongation, amphibian gastrulation, *Drosophila* germ-band extension and so on. Their mechanisms of cell intercalation do not seem to be universal. Apical faces of epithelial cells in *Drosophila* germ-band are polygonal and myosin II is enriched in specific cell boundaries and the boundaries are contracted, while mesenchymal cells in amphibian gastrulation have spindle shape and active protrusions form at both tips.

Here we have elucidated mechanisms of two planar cell intercalations, cell border remodeling (e.g., *Drosophila* germ-band) and cell shuffling (e.g., amphibian gastrulation). We have found a general principle, "orientated cell extension" that works in the two cell intercalations. Furthermore we have proposed methods to distinguish them, which are useful for experimental studies of morphogenesis in animal development.

- 3P342 低酸素誘導因子核移行の酸素濃度依存性
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Oxygen dependency of nuclear translocation of HIF-1α in human cell line
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Hypoxia causes adaptive responses due to hypoxia-inducible factor-1α (HIF-1α) which is degraded by ubiquitin-proteasome through HIF-1α prolyl hydroxylase (PHD) under normoxic conditions. In recent papers, when cells were exposed to 1 % O₂, HIF-1α became stable and localized to nucleus, whereas the purified PHD hydroxylated proline residues of HIF-1α in O₂ < 0.1 %. This discrepancy may be explained by factor(s) other than PHD in the HIF-1α pathway. To understand the pathway in detail, the nuclear translocation of HIF-1α was determined in HeLa cells with or without transfection of a plasmid containing HIF-1α gene fused to green fluorescent protein (GFP-HIF-1α). When cells without transfection were exposed to 1 % O₂ for 24 hr, a remarkable increase in expression of intrinsic HIF-1α was shown by the western blot. Then, after reoxygenation for 48 hrs, heme oxygenase-1 was induced, suggesting that one of the target genes of HIF-1α was at least activated transcriptionally and the translation followed. In cells after 3 days of transfection, fluorescence was frequently observed in cytosol. When the transfected cells were treated with 0.4 mM cobalt chloride that inhibits PHD, number of cells with fluorescent nucleus tended to increase. We will determine and report the oxygen dependency of the nuclear translocation with hypoxic incubator. When oxygen dependency of nuclear translocation of GFP-HIF-1α is equal to that of intrinsic HIF-1α, the fluorescence signal may be a clue to the protein interaction with the transport system.

- 3P341 低酸素誘導因子の内在性アンチセンス RNA の蛍光測定
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Detection of natural antisense transcript of hypoxia inducible factor-1α using fluorescence spectroscopy
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When oxygen supply to cells decreases, hypoxia inducible factor-1α (HIF-1α) regulates expression of various target genes for adaptation. Under normoxic conditions, HIF-1α is subjected to ubiquitin-proteasome degradation process through hydroxylation of proline residues with HIF-1α prolyl hydroxylase (PHD). In addition to HIF-1α pathway with PHD, it was reported that natural antisense transcript of HIF-1α (HIF-NAT) may alter the ratio between the two spliced forms of HIF-1α. However, it is unclear whether HIF-NAT expression and sense-antisense hybridization depend on the oxygen concentration. In the present study, HeLa cells were transfected with plasmids containing HIF-NAT or HIF-1α mRNA gene fused to green fluorescent protein (GFP) gene. In each plasmid, fluorescence was observed in transfected cells. When cells were exposed to 1 % O₂, expression of HIF-1α mRNA remained unchanged. Besides the RNAs expression, hybridization between HIF-NAT and HIF-1α mRNA would be an important step for translation. The hybrid was measured by fluorescence correlation spectroscopy (FCS). In the phantom experiment with complementary sequences of HIF-NAT and HIF-1α mRNA, we detected most hybrid under the conditions, where molar ratio of template to probe was 5. This suggests that the hybrid detection by FCS would be sensitive to the ratio. We will report the expression of the RNAs and the hybridization in transfected cells placed in a hypoxic incubator changing from 21% to 1% O₂.

- 3P343 線虫体壁筋タンパク質の量と時期を制御している転写因子の単離同定
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- 3aK10 Isolation and characterization of transcription factors controlling quantity and timing of the body wall muscle proteins in *C. elegans*
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MyoD homolog of *Caenorhabditis elegans* is important but not essential for the body-wall muscle gene expression. Three enhancer sequences of the CeMyoD gene, *hlh-1* are isolated in the second intron of the gene. To isolate the regulatory factor of body-wall muscle gene expression, which determines quantity of muscle proteins and time of muscle formation, we performed a modified-yeast one-hybrid screening and isolated LIM-8 and CeTIS-11 that show homology to TRIP6/Zyxin and TIS-11, respectively. LIM-8 interacted with CeMyoD but did not bind to enhancer sequences directly. A series of *lim-8::gfp* fusion genes were expressed in body-wall, vulval and anal muscles, and other non-muscle tissues. Anti-LIM-8 stained the locus of non-overlapping to DAPI staining DNA region in nucleus of body wall muscles. CeTIS-11 was also located body wall muscles and other tissues. *lim-8* (RNAi) or CeTIS-11 (RNAi) worm decreased to 80 % or 18% of the mobility of non-treated animal. CeTIS-11 (RNAi) worm also show egg-laying defect that comes from loss function of vulva muscle. This was the difference between LIM-8 bound to CeMyoD and CeTIS-11 directly bound to 1275 enhancer of the CeMyoD gene. These results suggest that LIM-8 regulates the body-wall muscle gene expressions interacting with CeMyoD. We propose an expression control model for the body wall genes.