Embryogenesis of mammals is a process during which the developmental potential of embryo's cells is gradually reduced. Fertilization of the oocyte initiates the formation of a one-cell embryo - the zygote, which undergoes a series of mitotic divisions. The resulting cells (blastomers) gradually lose their developmental potential, differentiate and create all cell lineages, which, in the postimplantation development, will contribute to the body of the fetus, placenta and fetal membranes. Around the time of implantation mouse embryo becomes a blastocyst, which is composed of outer layer of trophectoderm (TE), which will form the embryonic part of the placenta, and inner cell mass (ICM). In the E3.5 blastocyst ICM consists of mixed precursor cells of primitive endoderm (PE) and epiblast (EPI). The layer of PE, which during further development is localised on the surface of ICM, will become a part of the yolk sac. The EPI cells, which are located in the middle of ICM, will create the body of the fetus and the majority of fetal membranes.

One of the methods used to determine the developmental potential of cells is the formation of chimeric aggregates of two embryos or parts of embryos that integrate and form a single embryo. In the 1960s of the last century Andrzej K. Tarkowski from the Department of Embryology at the University of Warsaw revealed that aggregation of two early mouse embryos results in the formation of a single blastocyst, which after the transplantation to the female reproductive tract, develops into a normal and fertile offspring. This experiment has provided that the preimplantation mammalian embryo, in contrast to the embryos of other vertebrates, as well as invertebrates, is characterized by the ability to regulate its own development in response to an experimental change in the number of its cells. The cells of the embryo communicate with each other and "sense" this excess or deficiency, as well as use different strategies to adapt and successfully continue the further development. This knowledge has been used in the preimplantation diagnosis for genetic testing of the embryos before transferring them to the mother's uterus, as well as it helped to explain the causes of monozygotic twin pregnancies, which are the result of division of a single fertilized oocyte into two separate embryos.

The mechanisms governing the plasticity and regulative nature of the mouse embryos are still not fully understood. One of the signaling pathways potentially involved in regulating the development of the mouse embryo may be the Fgf4/MAPK (*fibroblast growth factor 4/mitogen-activated protein kinase*) pathway. This pathway plays a key role in the process of cell differentiation in ICM, which leads to the arise of two cell lineages: EPI and PE. The aim of the current project is to investigate whether paracrine interactions involving Fgf4/MAPK signaling pathway regulate the development of mouse embryo and underlie the basis of its plasticity, manifested by the proper formation of all three cell lineages (EPI, PE and TE), which are necessary for the embryo to continue its postimplantation development within the mother's uterus. One of the strategies to demonstrate how the embryo regulates its development is the disruption of this development and the examination of the consequences of such manipulations. The tool in the planned experiments will be a chimeric embryo composed of two components which significantly differ in the developmental stage. To investigate whether both components communicate through Fgf4/MAPK transduction pathway and to check whether this signaling determines the successful development, I will experimentally disrupt the interaction between two components of the chimeric embryo and investigate the consequences of such manipulations. The experiment will consist in blocking the possibility of receiving a Fgf4 signal, secreted by the ICM cells, as a result of decreased expression of selected genes encoding proteins of the Fgf4/MAPK pathway by siRNA (*short interference RNA*) injected into the cytoplasm of the mouse zygotes. When the embryos reach the 8-cell stage, they will be aggregated with the ICMs of the E3.5 mouse blastocysts. Chimeric aggregates will be cultured *in vitro* until the blastocyst stage and after fixation the proportions of the progeny of the two components in all primary lines (EPI, PE and TE) of the chimeric blastocysts will be determined by immunofluorescence method using cell lineage-specific markers. By using embryos expressing red and green fluorescence proteins, the fate of both components in the chimeric embryos will be followed and time lapse recorded under the fluorescence microscope. Time lapse recording will allow to obtain films illustrating the development of chimeric embryos from the moment of aggregation until they reach the blastocyst stage. Analysis of such films, using appropriate software, will allow to track the location of single cells in the embryos.

Using a chimeric embryo as a model will allow us to extend the knowledge on regulative nature of the mouse embryo development, the model organism in the mammalian developmental biology.