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Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

# From blastomeres to somatic cells: reflections on cell developmental potential in light of chimaera studies – a review

**Krystyna Żyżyńska-Galeńska*, Anna Piliszek, Jacek A. Modliński**

Department of Experimental Embryology, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec, 05-551 Magdalenka, Poland

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**Mammalian development is a process, whereby cells from a totipotent zygote gradually lose their potency, i.e. their ability to differentiate, in the environment of the developing embryo. An ideal model for testing the real potential of cells is the experimental production of chimaeras. The first experimentally produced mammalian (murine) chimaeras were created by Tarkowski [1961] and since then many new methods of chimaera production have been developed, including injecting cells into the blastocyst's cavity or into cleaving embryos.**

**This review describes how different cell types, depending on the developmental stage or culture conditions, manifest their potential to contribute to chimaeras. Cell developmental potential has been analysed in pluripotent blastomeres, which can contribute to all embryonic and extra-embryonic lineages, albeit differently depending on their developmental stage. This is the case in blastocyst lineages, with various developmental potentials depending on the surrounding cells, and in more differentiated cells from different stages of pregnancy, which in some cases may colonise chimaeric animal tissue. Cell potential has also been analysed in embryonic stem and embryonal carcinoma cells, which are pluripotent and efficiently contribute to chimaeras; in multipotent fetal and adult stem cells, which can also participate in chimaera formation; and in somatic mouse embryonic fibroblasts (MEFs), which can also be reprogrammed in the environment of the cleaving embryo. Interspecies chimaera studies have also demonstrated the pluripotency of foreign cells. Experiments with chimaeras have shown that not only pluripotent embryonic cells are capable of contributing to chimaeras, so are adult cells, which plasticity is now well-documented.**

**KEY WORDS: adult stem cells / cell plasticity / chimaera / developmental potential / pluripotency / reprogramming of somatic cells**

*Corresponding author:k.zyzynska@ighz.pl

The development of every mammalian organism begins with the formation of a totipotent zygote. During the course of development, subsequently formed cells lose their potency, defined as the ability to further differentiate. This phenomenon, described by Waddington as the "epigenetic landscape" [Waddington 1957], may be observed during development as the consecutive emergence of increasingly differentiated cells. However, based solely on observations of an unperturbed development it is impossible to discern between cells' innate potential and the influence of the neighbouring cells. Real evidence comes from studies using experimentally produced chimaeras, when cells are forced out of their original environment. This type of experimental setup reveals that their abilities and behaviour during development depend also on the influence of the environment, or the "niche" they are placed in.

A chimaera is an organism consisting of at least two different genetic backgrounds, derived from two different zygotes [McLaren 1972]. Natural chimaerism is a rare phenomenon that occurs only within species. However, in the early 1960s we gained a new powerful tool to understand the developmental potential of embryos, their plasticity and their components' capabilities: man-made chimaeras, which may be created both within and also between species. The first experimentally produced mammalian (murine) chimaeras were born in March 1961 at the Department of Zoology, University College of North Wales, U.K. and were created by a Polish embryologist Andrzej K. Tarkowski. The results of this experiment were soon published in *Nature* [Tarkowski 1961]. One year later Beatrice Mintz also described successful attempts to produce chimaeric mouse blastocysts [Mintz 1962] and then the births of viable, overtly chimaeric mice [Mintz 1965]. In both experiments chimaeric mice were produced by aggregating two cleavage stage embryos (at the same developmental stage), denuded of *zona pellucida* and cultured together. Following this treatment aggregated embryos surprisingly developed into one blastocyst, giving rise to animals containing components from both original embryos. This type of chimaera, composed of aggregated embryos, is called an aggregation embryonic chimaera.

Since 1961 the original method of aggregating cleaving embryos has been significantly modified and different microsurgical techniques of producing chimaeric embryos/animals have been developed. Chimaeras may be produced not only by aggregating two (or more) cleaving embryos, but also by combining a "normal" embryo with: (1) embryonic cells of asynchronous age; (2) parthenogenetic embryos; (3) embryonal carcinoma (EC) cells; (3) embryonic stem (ES) and embryonic germ cells; and (4) recently also somatic cells (see below). Methods for generating chimaeras are shown in Figure 1, while types of donor cells are shown in Figure 2.

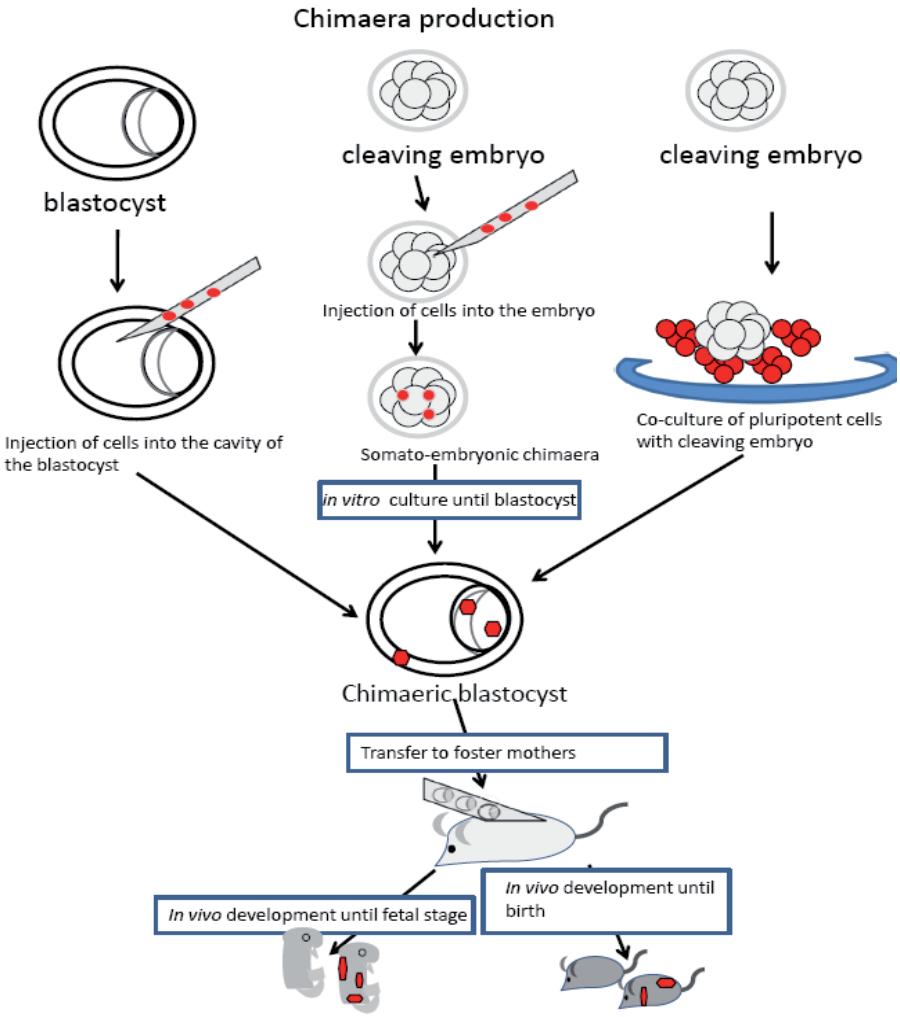


Fig. 1. Methods of chimaeras' generation. Red color symbolizes donor marker.

**Developmental potential of blastomeres from cleaving embryos**

In addition to thousands of viable chimaeric mice being born following the aggregation of intact cleaving embryos, aggregation embryonic chimaeras have also been generated in other laboratory mammals, specifically in the rat [Mayer and Fritz 1974] and rabbit [Gardner and Munro 1974], as well as farm animal species such as sheep [Willadsen and Fehilly 1983; Butler *et al.* 1987] and the pig [Anderson *et al.*

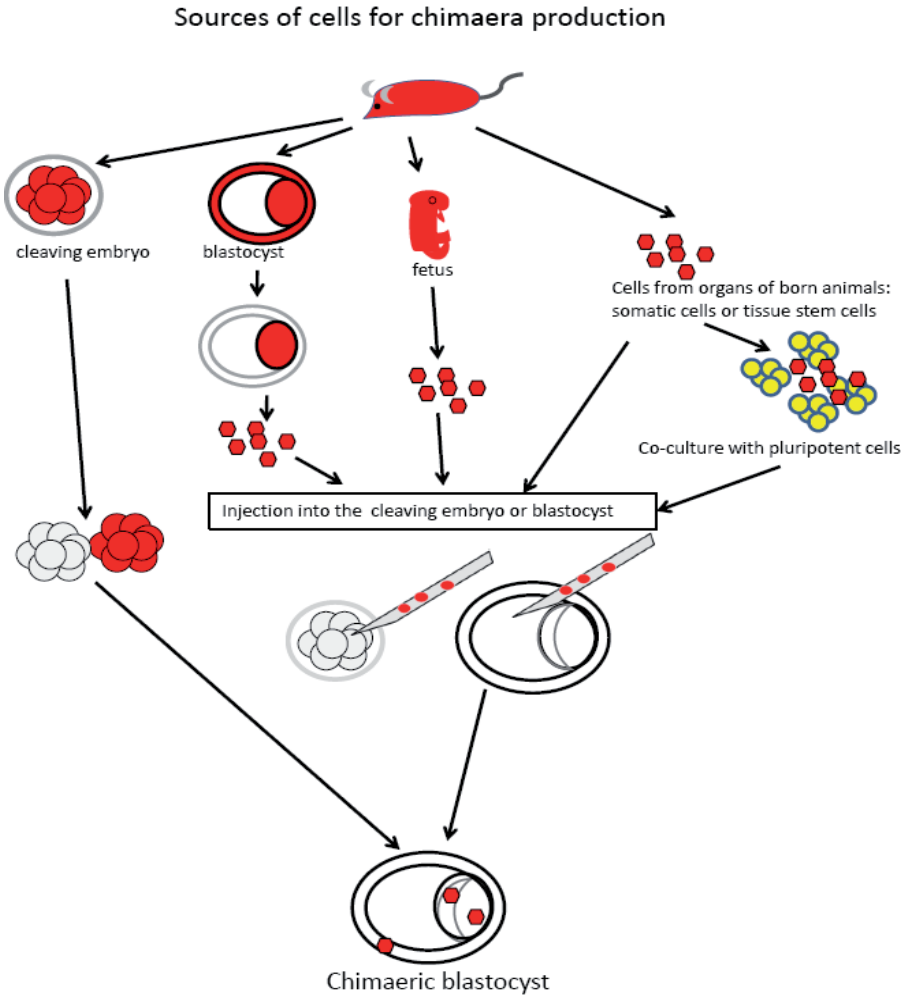


Fig. 2. Sources of cells for chimaera production. Red color symbolizes donor marker.

unpublished, cited in Yang and Anderson 1992]. When whole embryos of the same developmental stage are aggregated, the descendant cells of the two components of the resulting chimaera usually have (or should have) an equal, or similar, chance to participate in the formation of the chimaeric inner cell mass (ICM). These potentially uneven chances may result from different rates of embryo cleavage (genetically determined), from slightly different developmental ages of the two types of blastomeres, or from the fact that different cells within an embryo have unequal developmental potential (due to differentiation).

Chimaeras have been an indispensable tool for analysing lineage specification in mammalian embryos. At the 16-cell stage the first two lineages of the cleaving embryo emerge, i.e. unpolarised inside cells and polarised outside cells surrounding the first group. Injection of 1/16 blastomeres into an 8-cell stage morula [Rossant and Vijh 1980] has shown that outside cells of 16-cell stage embryos retain the ability to contribute to both inside and outside compartments in the morula (and, consequently, to the ICM and trophectoderm and their derivatives). By aggregating chimaeras from fluorescently labelled inside and outside blastomeres, Suwińska and collaborators have confirmed that both inside and outside cells of 16-cell embryos are able to contribute to fetal development to term, proving them to be totipotent. The developmental potential of 32-cell stage outside blastomeres is strikingly different: they can only contribute to the trophectoderm, and after aggregation with each other they form trophoblastic vesicles. On the other hand, inside cells of the same stage are still capable of contributing to the trophectoderm and ICM, but are unable to facilitate fetal development alone: the support of outside cells is necessary [Suwińska *et al.* 2008].

Some studies suggest that in intact embryos, blastomeres that cleave faster than others preferentially colonise the ICM rather than the trophectoderm [Piotrowska-Nitsche and Żernicka-Goetz 2004, Piotrowska-Nitsche *et al.* 2005, Plusa *et al.* 2005]. This has been confirmed by experiments of asynchronous aggregations of embryos/blastomeres. Spindle [1982] analysed the blastomere (labelled at the 2-cell stage with $^{3}$[H]thymidine') allocation in preimplantation mouse chimaeras obtained after aggregation of 4- and 8-cell stage embryos. Her results suggest that blastomere allocation in preimplantation mouse chimaeras is not a random process and that blastomeres of embryos developing ahead of others preferentially colonise the ICM. Furthermore, non-chimaeric lambs were obtained after aggregation of a single 1/8 sheep blastomere with a 1/4 goat blastomere and subsequent transfer of chimaeric embryos into female goats [Fehilly *et al.* 1984]. Similarly, following the aggregation of two 1/8 goat blastomeres with a single 1/4 sheep blastomere the chimaeric embryos were transferred to recipient ewes, which resulted in the births of non-chimaeric kids [Meinecke-Tillman and Meinecke 1984].These observations indicate that the more advanced blastomere partner contributes more cells to the ICM and later, to the embryo proper, than the less advanced one.

On the other hand, contrary evidence has also been presented. Prather and First [1987] aggregated single 1/8 murine blastomeres with intact 2-cell embryos and found that the descendants of 1/8 blastomeres form a "patch area" and do not preferentially migrate to the ICM. According to those researchers, "*possible mechanisms for this phenomena are that the eight-cell blastomere is physically excluded from the ICM by position or polarization, or that it is differentiating ahead of the two-cell component and becomes trophectoderm*".

Embryos that are normally unable to develop beyond early stages of development may be rescued in the so-called "rescuing chimaeras", in which the normal (i.e. diploid zygotic) component enables their prolonged survival. The best examples

are "rescuing diploid/diploid chimaeras" created from the normal and from parthenogenetic components [Stevens *et al.* 1977, Surani *et al.* 1977, Stevens 1978], as well as gynogenetic [Anderegg and Markert, 1986] and androgenetic embryos/blastomeres [Surani *et al*. 1988, Mann and Stewart 1991]. It has also been shown that diploid-triploid mouse "rescuing chimaeras" may develop up to adulthood and that 2N/3 N chimaerism was noted in the liver, intestines, heart and lungs [Suwińska *et al.* 2005]. Another example of "rescuing chimaeras" could be the embryos, in which one of the two 1/2 blastomeres was enucleated and a somatic cell's karyoplast was injected into it, which has been shown to produce at least one normal rabbit chimaera [Skrzyszowska *et al.* 2006]. Another study suggests that aggregation of cloned embryos may improve cloning efficiency [Buemo *et al.* 2016]. In this study three porcine embryos were aggregated to obtain one blastocyst. The author suggest that positive effects of embryo aggregation might be either due to (a) the epigenetic compensation of chimaera components, (b) an increase in embryo cell number, (c) the microenvironment generated by cell interaction, or (d) a combination of all those situations. Taken together, these observations suggest that chimaeric complementation (aggregation) enhanced the developmental potential of cloned embryos.

Another type of rescue is possible by the aggregation of diploid components: 2N embryos [James *et al.* 1995, Mackay and West 2005], 1/16 or 1/32 blastomeres [Tarkowski *et al.* 2010], and ES cells with tetraploid embryos or blastomeres [Nagy *et al.* 1990]. In 2N/4N embryos the tetraploid cells contribute to and survive in the fetal membranes, but are gradually eliminated from the embryo proper [Tarkowski *et al.* 1977, James *et al.* 1995, Mackay and West 2005].

**Developmental potential of blastocyst-stage cells**

Differences in the developmental potential of blastocyst stage cells have been also confirmed using the chimaera approach. Dissociated cells of 4.5 days *post coitum* (d.p.c.) mouse ICM were divided into classes based on their morphology (rough, smooth, intermediate), and injected into host blastocysts [Gardner and Rossant 1979]. Analysis of the resulting fetuses showed that each of the injected cells was able to colonise only one of the fetal compartments, either embryonic tissues (smooth cells) or extraembryonic membranes (rough cells), thus proving that the rough (primitive endoderm) and smooth (epiblast) cells have distinct fates at this point.

A more detailed study using the fluorescent marker of cell fate specification (Pdgfra-H2B-GFP) analysed changes in the potential of primitive endoderm (PrE) and epiblast (Epi) cells during consecutive stages of mouse blastocyst development. Grabarek *et al.* [2012] showed that Epi cells are more committed to their own blastocyst layer, whereas plasticity of PrE cells allows them to change their fate to all the three blastocyst lineages: trophectoderm, EPI and PrE.

**Developmental potential of ES and EC cells**

The method of chimaera creation was further improved by the technique of microsurgical cell injection, which was first used to introduce ICM cells into the blastocyst cavity [Gardner 1968]. Gardner's technique, which was very sophisticated and required the use of five different micromanipulation tools, was soon simplified by Moustafa and Brinster [1972a,b] and later by Babinet [1980]. Babinet's method required only two instruments: a holding pipette and a specially prepared injection pipette. Injection chimaeras soon became a widely used tool to test the developmental potential of various cell types. The generation of viable chimaeric mice (which could survive until adulthood) containing descendants of cells derived from EC cells [Brinster 1974, Mintz and Illmensee 1975, Papaioannou *et al*. 1975], from ES cells [Bradley *et al.* 1984, Beddington and Robertson 1989] and from primordial germ cells [Stewart *et al.* 1994] unequivocally proved their pluripotency.

The potential of ES cells was also demonstrated by Stewart [1982] based on his method of co-culturing blastocysts with ES or EC cells. The same method has been used to create mice from induced pluripotent stem cells (iPSCs), proving that they have a developmental potential comparable to that of ES cells [Takahashi and Yamanaka 2006, Wernig *et al.* 2007].

In addition to blastocysts, other preimplantation stage embryos may also serve as recipients in injection chimaeras. Another possibility, developed later, was connected with the injection of cells into the perivitelline space, under the *zona pellucida* of 8-cell cleaving embryos [Rossant and Vijh 1980, Thomson and Solter 1988]. In these experiments pluripotent cells were incorporated into an embryo and the resulting chimaera continued developing until birth. These 8-cell recipient embryos were also used to confirm the ability of ES cell lines to contribute to chimaeras. Tokunaga and Tsunoda [1992] showed that ES cells produce chimaeras at a high rate (80%), while Saburi [1997] showed that ES cells injected into 8-cell embryos are predominantly incorporated into the ICM.

**Developmental potential of fetal cells from various stages of pregnancy**

The idea of producing chimaeras consisting of cells from different stages of development was introduced by Moustafa and Brinster [1972b], who injected fetal cells 5.5, 8 and 12 d.p.c. into blastocysts. Although cells from 5.5 d.p.c. contributed to chimaeras, which was confirmed in 15- to 17-day-old fetuses, only in a few cases were cells from the 8$^{th}$ d.p.c. found in chimaeras and no progeny of 12 d.p.c. cells were found in chimaeras. These results seem to be consistent with the results of Gardner *et al*. [1985], who showed that the oldest cells from primitive ectoderm (fetal tissues) that could contribute to chimaeras were 5 d.p.c. No coat colour chimaeras were obtained from cells from the 6$^{th}$ or 7$^{th}$ d.p.c. Brinster [1974] attempted to obtain chimaeras by injecting bone marrow cells of CBA-T6T6 mice and teratocarcinoma

cells of 129 SCSl mice. Only in the latter case was one out of the 60 animals born with a coat colour chimaera. No CBA-T6T6 cells were found among 2,000 chromosome spreads. However, when he grafted the skin of CBA-T6T6 mice to the first group, a significantly longer survival time was observed.

**Adult and fetal stem cell plasticity**

It was not only fetal cells that turned out to have the potential to populate chimaeras after blastocyst injection. Other studies showed that mouse haematopoietic stem cells, when injected into blastocysts, could contribute to chimaeras and they were found in the yolk sac, fetal liver and peripheral blood, as well as the bone marrow of adult animals [Geiger *et al.* 1998]. Neural stem cells (NSCs) have been reported to contribute to all the three germ layers (ectoderm, endoderm and mesoderm) of embryos [Clarke *et al.* 2000], when injected into chick embryos, mouse blastocysts or morulas. The authors suggest that NSCs have a broad developmental capacity when exposed to the embryonic environment. Another research team analysed the developmental potential of NSCs after injection into blastocysts in mouse fetuses and adult animals [Harder *et al.* 2004]. In fetuses, NSCs progeny were found in haematopoietic tissues, whereas in adult animals most NSC progeny were found in neural tissues. Furthermore, Jiang *et al.* [2002] showed that single multipotent adult progenitor cells (MAPCs) isolated from the bone marrow could differentiate into most types of somatic cells from all the three germ layers. Kues *et al.* [2005] isolated a subpopulation of fetal somatic stem cells that after culture in a high serum concentration and injection into blastocysts contributed to the liver, genital ridge and tongue tissues of chimaeric fetuses from 5.5 d.p.c. A population of mouse CD34+ bone marrow cells has been reported to have the capacity to differentiate to organs of all the three germ layers after injection into blastocysts [Pessac *et al.* 2012]. Another group analysed the potential of porcine skin-derived progenitors (SKPs) derived from the neural crest, as well as porcine NSCs and fibroblasts derived from SKPs [Zhao *et al.* 2010, Zhao *et al.* 2012]. The results indicate that SKPs, when injected into 4- to 8-cell embryos, can integrate with the blastocyst and can populate organs of all the three germ layers. However, in these experiments NSCs as well as SKP-derived fibroblasts failed to contribute to chimaeric piglets. These examples suggest that adult or fetal stem cells apparently have a broader developmental potential than just populating their original tissue.

**Reprogramming of non-stem somatic cells in chimaeras**

The first observations of non-stem cells integrating with cleaving embryos were made by Burnside and Collas [2002], who injected ES cells, HC-11 epithelial cells and NIH3T3 fibroblasts into 4- to 8-cell stage embryos. All the three types of cells adhered to blastomeres, but only the ES cells and HC-11 epithelial cells formed gap junctions with blastomeres within 24 hours of culture. The authors concluded that in

HC-11 cells, *Oct3/4* expression is activated by cAMP signalling. Only when NIH3T3 fibroblasts were reprogrammed by HC-11 cell extracts, did these cells form gap junctions with blastomeres and activate *Oct3/4*.

Further studies of chimaeras with fibroblast compounds were performed in our laboratory. Piliszek *et al.* [2007] showed that mouse embryonic fibroblasts (MEFs) injected into the centre of an 8-cell stage embryo could integrate with that embryo. At the blastocyst stage, these cells populated all the three blastocyst lineages. Mid-term fetuses were analysed for the presence of the MEF cells marker, glucose phosphate isomerase (GPI). Both donor and recipient isozyme GPI-1A and GPI-1B were found in only one, delayed fetus; otherwise, donor isozymes were found only in fetal membranes. However, the hybrid isozyme GPI-1AB was found frequently in fetuses and fetal membranes, which indicated fusion of the donor fibroblasts with recipient cells. In animals that were born, no coat colour chimaerism was observed. Organs from these same animals were also analysed for the presence of GPI isozymes. Hybrid isozyme GPI-1AB and the donor isozyme GPI-1B were found in organs from all the three germ layers.

Chimaerism of hybrid cells with a host embryo was also confirmed by Lee *et al.* [2013], who showed that MEFs co-cultured with ovarian cells may be reprogrammed. MEFs aggregated with less differentiated cells and became colony-forming fibroblasts (CFFs), which visually resembled ES cell-like colonies. CFFs produced somatic chimaeras by (1) co-culture with blastocysts or (2) injection into blastocysts. However, CFFs were aneuploid with a tetraploid-like chromosome number and therefore no germline transmission was observed. This study confirmed that a specific niche can induce somatic cell reprogramming into stem cell-like cells.

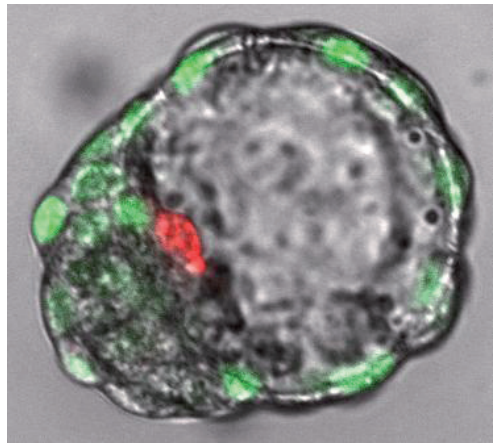

Fig. 3. Chimaeric blastocyst, obtained by injection of mouse embryonic fibroblast (MEF) cells expressing red fluorescent protein (RFP) into a cleaving embryo. The recipient embryo was transgenic for the expression of green fluorescent protein (GFP) in the nuclei of all cells. The RFP-expressing cell was integrated with primitive endoderm (PrE), which was later confirmed by expression of the PrE marker, Gata4.

Our further studies with the use of fluorescence markers (red fluorescent protein, RFP, green fluorescent protein, GFP) revealed that fibroblasts injected into cleaving embryos could be reprogrammed within two days of culture (Fig. 3). This reprogramming was confirmed by expression of the blastocyst lineage markers, Nanog, Gata4 and Cdx2 [Żyżyńska *et al*. 2013]. Use of live fluorescent markers allowed us to confirm that MEFs could be reprogrammed in somatic-embryonic chimaeras both by fusion with the host embryo and without fusion.

In fetuses and fetal membranes, progeny of injected cells of both fused and non-fused origin were found (data not published). In animals that were born, both fluorescence and genetic donor markers were detected in organs of all the three germ layers, among diploid cells.

In a similar manner, the potential of sheep fetal fibroblasts was assessed by injection into ovine blastocysts. Three lambs (of 7 born) derived from fetal fibroblast-injected blastocysts at birth had areas of pigmentation (dark skin of the nose and lips, some dark hair nearby, dark hooves). When samples from organs of all the three germ layers were analysed on the genetic level, in two analysed lambs donor markers were found in organs of all the three germ layers [Karasiewicz *et al.* 2008].

**Interspecies chimaeras**

The aggregation/injection technique also facilitates the creation of interspecies chimaeric animals. Mystkowska [1975] constructed bank vole (*Myodes glareolus*)-mouse (*Mus musculus*) chimaeric aggregation embryos and analysed their development (after transfer to mouse recipient females) both before and after implantation. The most advanced chimaeric embryos developing until the 9$^{th}$ and 10$^{th}$ day of pregnancy were alive, morphologically normal and their chimaerism was confirmed by karyological analysis. The first report on the completely normal development of interspecies chimaeras in mammals was published by Rossant and Frels [1980]. In their experiments, *Mus caroli* (a wild species from Asia) ICM cells were injected into *Mus musculus* blastocysts. Although both species showed multiple genetic differences, differed in the timing of preimplantation development (in *M. caroli* preimplantation development is complete up to 20 hours earlier than in *M. musculus)* and in the duration of the gestational period, and although they do not interbreed in nature, the *M. musculus-M. caroli* chimaeras survived after birth and developed normally. They also found that *M. musculus-M. caroli* chimaeras and *M. musculus-M. musculus* control chimaeras showed striking similarities in their somatic tissue composition. There was thus no evidence to suggest that *M. caroli* cells were selected against *M. musculus* cells.

Later the successful development of interspecies chimaeras to adulthood was observed in sheep-goat [Fehilly *et al*. 1984, Meinecke-Tilllman and Meinecke 1984, Członkowska *et al.* 1988] and in two types of cattle, *Bos taurus* and *Bos indicus* [Summers *et al.* 1983, Williams *et al.* 1990]. Fehilly and Willadsen [1986] reported the birth of lambs (which were clearly sheep-cow chimaeras, with manifold malformations)

generated by aggregating 1/4 sheep and 1/8 cow blastomeres. These results indicate that in some cases the generation of interspecies chimaeras can remove the reproductive barrier between species and that the mechanisms responsible for blastocyst formation can successfully operate across species boundaries [for a comprehensive review of interspecies chimaeras see: A.L Bonnicksen's "Chimaeras, Hybrids, and Interspecies Research" Georgetown Univ. Press, Washington D.C., 2009].

Harder *et al.* [2002] showed that it is also possible to create interspecies chimaeras using human adult stem cells. Following injection of human haematopoietic stem cells from cord blood into murine blastocysts, progeny of injected cells were found mostly in haematopoietic tissues (e.g. fetal liver and yolk sac) and in embryonic tissues. These progeny were also found in both the haematopoietic and nonhaematopoietic tissues (e.g. peripheral blood, thymus and spinal cord) of animals that were born. The use of human cells in generating interspecies chimaeras opened a new field of research, making it possible to create humanized organs for medical tests and potentially produce them in the future for transplantation medicine purposes.

Recently, Wu *et al.* [2017] generated live rat-mouse chimaeras with an extensive contribution from naïve rat pluripotent stell cells (PSCs) injected into mouse blastocysts. Rat-mouse chimaeras developed into adulthood and exhibited normal appearance and physiology. Moreover, they also showed that after injection of an intermediate type of human induced pluripotent stem cell (hiPSC) into pig blastocysts hiPSCs were able to integrate and subsequently differentiate in host pig post-implantation embryos.

## Conclusions and future challenges

This manuscript discusses how various cell types of various cell potency status are capable of contributing to chimaeras. In the first chapter we showed that blastomeres of cleaving embryos are totipontent, which means that they can contribute to all embryonic and extra-embryonic lineages. However, some authors have shown that in an experimental chimaeric design blastomeres that cleave faster may preferentially contribute to embryonic tissues. First differentiation occurs at the morula stage, when outside cells become precursors of extraembryonic lineages and inside cells maintain pluripotency. Experiments with the so-called rescue chimaeras have shown that even 1/32 blastomeres can support development with the assistance of tetraploid cells.

At the blastocyst stage further differentiation occurs. In ICM two lineages of cells emerge: PrE and Epi, of which only the latter is destined to contribute to embryo proper. However, in the specific chimaeric environment PrE cells turned out to be more plastic than EPI cells.

ES cells derived from blastocysts and EC cells from embryonal carcinoma are pluripotent, which means that they can contribute to all embryonic lineages. In chimaeras they preferentially populate ICM and later embryonic tissues. Adult stem cells are considered to have less potential than ES cells. However, recently a growing body of evidence has been presented that in chimaeras such cells can be plastic and

reprogram to retain the capability to contribute tissues of all the three embryonic lineages.

The final stage of differentiation is represented by somatic cells, which in normal circumstances remain a part of their own tissue. However, when injected to early stage embryos these cells can also be reprogrammed, not only by fusion with recipient blastomeres, but also by the influence of the embryonic environment.

The last paragraph shows that also interspecies chimaeras may be produced by aggregation or injection of cells of different origin. In various studies chimaeras of two different mouse species, as well as sheep-goat and rat-mouse chimaeras were obtained.

Numerous studies have shown that chimaeras are a good model for testing cell developmental potential as well as its reprogramming mechanisms. While previously only embryonic cells were considered pluripotent, now the plasticity of adult cells has been well documented. The contribution of progeny of injected cells to chimaeras depends both on these cells' potential and on niche properties that influence reprogramming. Adult stem cells frequently activate the expression of markers of surrounding cells in the chimaeric environment and contribute to all the three germ layers. While they do not naturally express these markers, somatic cells, e.g. fibroblasts, may also be reprogrammed not only by genetic manipulations [Takahashi and Yamanaka 2006], but also through the influence of cell extracts [Burnside and Collas, 2002] or even by contact with blastomeres in specific niches created by the early embryo.

Only a small fraction of reprogrammed cells is found in embryonic-somatic chimaeras, which leaves the question if all somatic cells have the ability to reprogram. Understanding the mechanisms, by which cells retain plasticity or undergo reprogramming, is yet to be clarified.

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