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Where do we stand now? – mouse early embryo patterning meeting in Freiburg, Germany (2005)

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Freiburg Meeting Participants (in alphabetical order): Vernadeth Alarcon, Jens-Erik Dietrich, Toshihiko Fujimori, David Glover, Takashi Hiiragi, Nihan Kara, Sophie Louvet-Vallee, Marek Maleszewski, Yusuke Marikawa, Bernard Maro, Nami Motosugi, Hitoshi Niwa, Zbigniew Polanski, Berenika Plusa, Davor Solter, Yayoi Toyooka, Akiko Tsumura, Masamichi Yamamoto, Magdalena Zernicka-Goetz. (Note: Richard Gardner did not join the meeting, but was of course invited to participate.) The invited moderators of the meeting were (in alphabetical order): Chris F. Graham, Jacek Kubiak and Andrzej K. Tarkowski.

Note from the Editor-in-Chief: This *Developmental Perspecitve* paper presents the reflections of its authors on the controversial subject of early embryo patterning in the mouse, on the basis of an international meeting, celebrated in Freiburg, Germany on 16th September 2005. It serves as a record of the meeting (though not strictly speaking, a Meeting Report), detailing the contentious issues that were discussed and highlights the unsettled nature of our current understanding of these issues.

Peer-reviewing of this manuscript elicited contradictory and somewhat passionate responses. It even came to light that a former version of this manuscript had not been considered to be suitable for publishing in a number of other prestigious journals in the field, due to claims of being appalingly imbalanced, misleading and even alarmist.

The mission of the *Int. J. Dev. Biol.* is to publish communications which throw light on our understanding of the mechanisms of development (see "Aims and Scope" of the journal), rather than cloud them over or generate fruitless controversies, in a manner respectful to all parties where controversial issues arise. Upon consultation with members of our Editorial Advisory Board, it was deemed both appropriate and timely to communicate, via this publication, differences in opinion which exist today in this area of research. In the confidence that the publishing of this paper will contribute in a healthy way to stimulating those experiments and collaborations which will resolve in an objective manner some of the currently contended issues outlined herein.

Juan Aréchaga (Editor-in-Chief)

Mechanism underlying mammalian preimplantation development has long been a subject of controversy and the central question has been if any "determinants" play a key role in a manner comparable to the non-mammalian "model" system. During the last decade, this issue has been revived (Pearson, 2002; Rossant and Tam, 2004) by claims that the axes of the mouse blastocyst are anticipated at the egg ("prepatterning model"; Gardner, 1997; Gardner, 2001; Piotrowska *et al.,* 2001; Piotrowska and Zernicka-Goetz, 2001; Zernicka-Goetz, 2005), suggesting that a mechanism comparable to that operating in non-mammals may be at work. However, recent studies by other laboratories do not support these claims ("regulative model"; Alarcon and Marikawa, 2003; Chroscicka *et al.,* 2004; Hiiragi and Solter, 2004; Alarcon and Marikawa, 2005; Louvet-Vallee *et al.,* 2005; Motosugi *et al.,* 2005) and the issue is currently under hot debate (Vogel, 2005). Deepening our knowledge of this issue will not only provide an essential basis for understanding mammalian development, but also directly apply to ongoing clinical practices such as intracytoplasmic sperm injection (ICSI) and preimplantation genetic diagnosis (PGD). These practices were originally supported by a classical premise that mammalian preimplantation embryos are highly regulative (Tarkowski, 1959; Tarkowski, 1961; Tarkowski and Wroblewska, 1967; Rossant, 1976), in keeping with the "regulative model". However, if the "prepatterning model" is correct, the latter will require critical reassessment.

On September 16, 2005 in Freiburg, Germany, many scientists (see "Meeting participants" above) who contributed to studies concerning mouse early patterning gathered and had an extensive discussion meeting in an attempt to find what may be the causes of the apparent discrepancy. While no solid answer was yielded, several critical issues were brought up that need to be

Abbreviations used in this paper: 2pb, second polar body; A-V, animal-vegetal (axis); ICM, inner cell mass; SEP, sperm entry position; TE, trophectoderm; ZP, zona pellucida.

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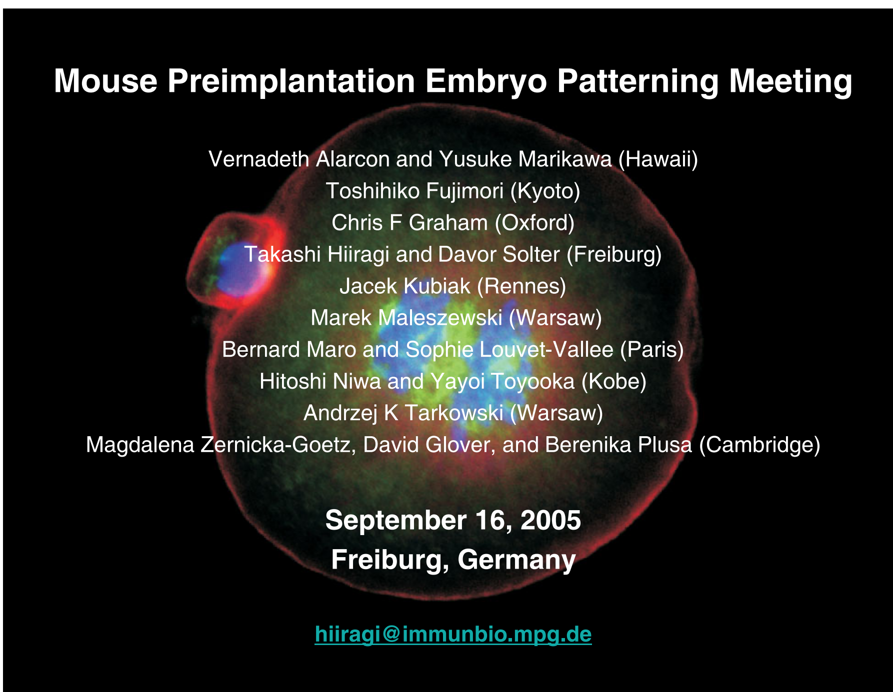
taken into consideration when performing further experiments to clarify the situation in future studies. In this paper, we summarize those issues point by point, which may hopefully serve as a guide for not only mammalian embryologists but also for non-mammalian scientists (Lawrence and Levine, 2006) when thinking of how our body is derived from a fertilized egg.



Poster of the Freiburg, Germany (2005) meeting

Oocyte polarity, sperm entry and first cleavage

In many organisms, polarity in the unfertilized oocyte, specifically "the animal-vegetal (A-V) axis", provides the first asymmetry on which the basic body pattern of the embryo is built (Gilbert, 2006). In sea urchins and frogs, for example, the formation of the germ layers is dictated by the factors which are differentially distributed along the A-V axis of the oocyte. Also, the entry of sperm, i.e., fertilization, is known to be critical for establishing further asymmetry in some animals. Namely, in ascidians, sperm entry triggers a series of cytoplasmic reorganization events which place cell fate determinants in specific areas of the zygote before the first cell division. In frogs, the entry point of sperm influences the orientation of cortical rotation, which ultimately determines the dorsal-ventral axis of the embryo. In these cases, oocyte polarity and the events prior to the first cell division play essential roles in embryonic patterning.

The key questions here are whether the mouse oocyte bears any hereditary asymmetry that is linked to embryo patterning and if the entry of sperm is involved in the establishment of any embryonic configuration. While these questions appear simple and straightforward, consensual answers have yet to be found. The conclusions of some studies apparently contradict those of others. Some of the controversial issues are centered on whether the sperm entry point (SEP) is associated with the polarity in the oocyte and with the first cleavage plane (see points ***a,c*** and ***d*** below), whether the "animal pole" can be identified by the location of the polar body (see point ***b***) and whether the orientation of the first cleavage plane is determined by intrinsic or extrinsic cues (point ***e***).

a. Preferential sperm entry into the oocyte

Zernicka-Goetz's lab initially reported that the first cleavage plane is specified as passing through both the sperm entry position (Piotrowska and Zernicka-Goetz, 2001) and the "animal pole", i.e. the site of the previous meiotic division, as identified by the location of the second polar body (2pb; Plusa *et al.,* 2002a). In their report (Piotrowska and Zernicka-Goetz, 2001), Zernicka-Goetz's lab showed that the sperm preferentially enters the "vegetal" third of the oocyte. In contrast, Hiiragi's lab found that it preferentially (63%) enters the half sphere of the oocyte overlying the meiotic chromosomes (Hiiragi and Solter, 2004; Motosugi *et al.,* 2006). This is one of the simplest discrepancies in the data itself and needs to be reassessed by other groups. *In vivo* fertilized egg can be recovered at 15-16 hours after the induction of superovulation by hCG injection and then the position of the fertilization cone, as a landmark of the sperm entry position, can be examined in relation to the 2pb. Exact determination of the SEP is critical with respect to assessing its relationship to the first cleavage. If the sperm preferentially enters around the animal pole, both Zernicka-Goetz's prediction (i.e., the cleavage passes near the animal pole and sperm entry point) and Hiiragi's prediction (i.e., it most likely passes between the animal pole and the sperm entry point; see point ***d*** below) are practically indistinguishable, because these two points are physically close. However, if the sperm enters at the vegetal half, these two models are incompatible.

b. Identification of the animal pole using the second polar body

The 2pb, a remnant of the last meiotic division, has been used as a stationary marker of the "animal pole" in several studies (Gardner, 1997, 2001; Piotrowska and Zernicka-Goetz, 2001; Piotrowska-Nitsche *et al.,* 2005; Piotrowska-Nitsche and Zernicka-Goetz, 2005; Plusa *et al.,* 2005). The "A-V axis" in the mouse egg was accordingly defined in a manner reminiscent to that of non-mammals. Zernicka-Goetz's group bases its observation on the premise that the 2pb does not change its position during early development. However, Hiiragi *et al.* reported that the 2pb moves towards the furrow during the first cleavage (Hiiragi and Solter, 2004), consistent with observations made by Richard Gardner´s laboratory (Gardner and Davies, 2003). In contrast, Zernicka-Goetz reported that the embryos do not show the 2pb movement during cleavage (Plusa *et al.,* 2005) and the first cleavage plane is specified by the midbody of the 2pb (Plusa *et al.,* 2002a). This discrepancy may be due to differences in experimental conditions (for example, deteriorated culture conditions can reduce the

dynamic movement of the 2pb). Also, a clear and objective definition of "movement", particularly the reference point of measurement, needs to be agreed upon among researchers.

c. Tracing of the sperm entry position (SEP) during cleavage

The model proposed by the Zernicka-Goetz team that the first cleavage plane is specified by the sperm entry position, is based on an experiment using fluorescent beads to mark the SEP (Piotrowska and Zernicka-Goetz, 2001). However, as pointed out previously (Davies and Gardner, 2002), the soundness of this method may require further verification. At the meeting, Hiiragi reported that beads attached to the cell surface move towards the cleavage furrow, indicating that the use of beads to trace the SEP may be ineffective and misleading. While the SEP has also been traced by different methods (Davies and Gardner, 2002; Plusa *et al.,* 2002b), its relationship with the first cleavage plane is still inconsistent.

d. The first cleavage plane in relation to the position of the two pronuclei

Hiiragi *et al.* reported that the first cleavage plane is specified as the plane between the apposing two pronuclei that have moved to the center of the egg (Hiiragi and Solter, 2004). In contrast, Zernicka-Goetz's lab reported that most of the first cleavage plane is perpendicular to the apposing plane of the two pronuclei and suggested that Hiiragi's results may be biased by cytochalasin treatment used during the experiments (Plusa *et al.,* 2005). However, this cannot be the sole cause of discrepancy, because cytochalasin is used only in one set of micromanipulation experiments and most of the data are based on the observation of embryos without cytochalasin treatment (Hiiragi and Solter, 2004). The discrepancy of the first cleavage specification in relation to pronuclei position may be due to differences in the conditions of time-lapse recording, such as the intensity of light exposure. Protocols for time-lapse observation under the physiological conditions need to be optimized and shared among researchers to resolve this type of discrepancy.

e. The first cleavage plane in relation to the shape of the egg

Data concerning the orientation of the first cleavage is widely agreed upon (Rossant and Tam, 2004) when the egg is under compression; cleavage takes place perpendicular to the long axis (Gray *et al.,* 2004), which is also in accordance with the pronuclei-apposition model (Hiiragi and Solter, 2004). Zernicka-Goetz's lab reported that the mouse egg is ellipsoidal with the sperm entry position located on its short-axis, with the first cleavage thus passing through the sperm entry position (Gray *et al.,* 2004). At the Freiburg meeting, Fujimori reported that the zona pellucida (ZP) is ellipsoidal before the first cleavage and the shape of the ZP is maintained until the blastocyst cavity expands, raising the possibility that the location of the first cleavage plane may also be affected by the external physical constraint.

Early cleavage and blastocyst axes

In embryos of many animal species, the plane of the first cleavage is spatially linked to a specific axis in the final body plan (Gilbert, 2006). For example, in the nematode *C. elegans*, the first cleavage plane is perpendicular to the anterior-posterior body axis, while in ascidians, it runs along the anterior-posterior axis and is orthogonal to the left-right body axis of the swimming larva. Such a distinct relationship between the first cleavage plane and the body axis is primarily due to the precise segregation of critical morphogenetic determinants between the first two blastomeres.

The question here is whether the mouse also exhibits any relationship between the first cleavage plane and the later body axis. While classic experiments demonstrated that both blastomeres of the two-cell stage mouse embryo are equally capable of giving rise to essentially any part of the final body, recent studies have given rise to an apparent contradiction. Some studies show a certain extent of association between the first cleavage plane and the embryonic-abembryonic axis of the blastocyst (Gardner, 1997, 2001; Piotrowska *et al.,* 2001). In this case, one blastomere of the two-cell stage embryo preferentially gives rise to the half side of the blastocyst where the Inner Cell Mass (ICM) is located, which implicates the presence and unequal segregation of morphogenetic determinants in the mouse zygote. On the other hand, other recent studies demonstrate the absence of relationship between the first cleavage plane and blastocyst axis (Alarcon and Marikawa, 2003; Chroscicka *et al.,* 2004; Motosugi *et al.,* 2005; Alarcon and Marikawa, 2005), thus corroborating the conclusions of the classic experiments (Tarkowski, 1959; Tarkowski, 1961; Tarkowski and Wroblewska, 1967; Rossant, 1976).

A number of current controversies are focused on whether the distribution pattern of two-cell stage blastomere descendants is influenced by the shape of the overlying ZP (***f,g*** below), what is the most effective and unbiased method to measure the clonal boundary of two-cell stage blastomere descendants (***h***), whether differences in mouse strains contribute to significant variation in blastomere behavior and capability (***i***), how to classify the orientation of the subsequent cleavage patterns when the apparent spatial landmark (i.e. polar body) is not always fixed in place (***j,k***) and how to define the embryonic-abembryonic axis when more than one blastocyst cavity appears at the early stages (***l***).

f. Preferential orientation of the blastocyst axis

Alarcon and Marikawa discussed (Alarcon and Marikawa, 2003) and Hiiragi's lab provided evidence for the mechanical constraint model (Motosugi *et al.,* 2005). In this model, the embryonic-abembryonic axis (identified by the location of the blastocyst cavity) is simply to be aligned along the long axis of the embryo, which may be imposed by the uneven shape of the ZP or experimental manipulations. This was confirmed by Fujimori at the Freiburg meeting. The preferential orientation of this axis in parallel with the longest diameter of the ellipsoidal ZP is statistically in agreement with the one reported by Gardner (Gardner, 2001). Importantly, if the two blastomeres at the 2-cell stage are aligned along the long axis of the ZP and if the embryo does not rotate within the ZP until formation of the blastocyst cavity, then the Em-Ab axis ends up perpendicular to the first cleavage plane. However, this may not be the case, as discussed below (see ***g***).

g. Rotation of the embryo within the Zona Pellucida

Hiiragi's lab reported that the embryo extensively rotates within the ZP after every cleavage during preimplantation development (Motosugi *et al.,* 2005), indicating that the marking of the ZP as a reference point for embryo orientation may be ineffective and misleading (Gardner, 2001). As presented at the meeting, some

groups (Fujimori and Maro) confirmed the extensive rotation of embryos within the ZP, while others (Zernicka-Goetz *et al.*) did not. Because this is of significant importance in light of the mechanical constraint model (see ***f***), further investigations are essential, particularly using standardized, rigorous criteria of measurement agreed upon among researchers.

h. Lineage analysis of the two-cell stage blastomeres

While some groups reported that the descendants of the two cell blastomeres tend to distribute either in the embryonic or in the abembryonic territory (Gardner, 2001; Piotrowska *et al.,* 2001; Plusa *et al.,* 2005), others reported not being able to reproduce this finding (Alarcon and Marikawa, 2003; Chroscicka *et al.,* 2004; Motosugi *et al.,* 2005). The cause of this contradiction may partly be due to differences among the groups in the methods used to analyze the distribution of blastomere descendants in the embryonic and abembryonic territories. Zernicka-Goetz defined the one-cell thick floor of the blastocyst cavity as the "boundary zone" that delineates the two territories of the blastocyst. Thus, when the number of blastomere descendants that cross the boundary zone and occupy the opposite territory is three or less, it was concluded that the blastomere was biased to give rise specifically to the embryonic or abembryonic territory. However, the boundary zone may be too large to be used as a means to distinguish between embryonic and abembryonic halves, because it is composed of nearly one third of the total cell number of the early blastocyst, playing a buffering role. Also, there is no rational justification for choosing the number of three or fewer cells as positive evidence for "predisposition" (Piotrowska *et al.,* 2001; Plusa *et al.,* 2005). As an alternative means to distinguish between embryonic and abembryonic halves, the "boundary plane" is used by Hiiragi´s lab (Motosugi *et al.,* 2005). This is defined as one minimizing the number of the blastomeres crossing over it (Motosugi *et al.,* 2005). There are also differences in the 2D (Piotrowska *et al.,* 2001; Plusa *et al.,* 2005) vs. 3D (Motosugi *et al.,* 2005) methods of analysis. It remains to be clarified if the discrepancy in the relationship between the boundary of the 2-cell blastomere descendants and that of the embryonic and abembryonic area can be explained by difference in methodologies.

Recently, Cdx2 has been proposed as a trophectoderm determinant whose mRNA is localized in the oocyte and becomes exclusively segregated into one of the two-cell stage blastomeres (Deb *et al.,* 2006). Despite the fact that this idea was published after the Freiburg Meeting, we feel obliged to discuss this paper briefly. The lineage tracing data presented in Deb *et al.* (2006) shows that one two-cell stage blastomere contributes solely to the ICM of the blastocyst, while the other blastomere contributes to the trophectoderm. This contradicts not only our observations (Alarcon and Marikawa, 2003; Chroscicka *et al.,* 2004; Motosugi *et al.,* 2005; Alarcon and Marikawa, 2005), but also those made by the researchers who support the "prepatterning model" (Gardner, 2001; Piotrowska *et al.,* 2001; Plusa *et al.,* 2005) and requires further investigation.

i. Mouse strain and culture media

The above-mentioned inconsistencies may be due to differences in experimental conditions such as mouse strain and culture media. Nevertheless, there is concern that such fundamental mechanisms are likely not to depend on strain or culture media. Here, information on strains and media is provided for clarification: Alarcon and Marikawa use F2 embryos obtained from intercrosses of the same F1 mice (B6D2 or B6CBA) or intercrosses of outbred CD-1 mice and modified FHM or modified KSOM-AA media (Ho *et al.,* 1995); the Hiiragi lab uses F2 embryos from B6C3 F1 females mated with males of B6C3 F1 or B6D2 F1 and KSOM-AA media (Ho *et al.,* 1995); the Fujimori lab uses embryos of B6 or 129 mice and KSOM-AA media (Ho *et al.,* 1995); the Zernicka-Goetz lab uses F2 embryos from B6CBA F1 mice mated with B6CBA F1 mice and KSOM-AA or FHM media; the Gardner lab uses embryos of PO mice and those obtained from intercrosses of CBAB6 F1 mice and MTF or KSOM media. Note that the same strain, specifically F2 embryos from B6CBA F1 mice, yielded the two conflicting conclusions, suggesting that strain difference may not be the sole source of the contradictions (Piotrowska and Zernicka-Goetz, 2001; Alarcon and Marikawa, 2005).

j. Pattern of the second cleavage

Piotrowska and Zernicka-Goetz (2001) reported that the 2-cell blastomere to divide early tends to give rise to the embryonic-hemisphere of the blastocyst, while the later-dividing blastomere tends to give rise to the abembryonic-hemisphere, implying that the difference in developmental potential between the two blastomeres is associated with the timing of the second cleavages. In contrast, such a tendency has not been observed by five other groups, namely Gardner (2001), Fujimori *et al.* (2003), Motosugi *et al.* (2005), Alarcon and Marikawa (2005) and Maleszewski (unpublished data).

While the Zernicka-Goetz group reported that a regular cleavage pattern exists at the 2- to 4-cell transition in most embryos (Piotrowska-Nitsche and Zernicka-Goetz, 2005), Louvet-Vallee *et al.* (Louvet-Vallee *et al.,* 2005) showed that the second cleavage pattern is random with respect to each other and to the first cleavage plane. At the Freiburg meeting, Plusa pointed out that the analysis of spindle orientation in the paper by Louvet-Vallee *et al.,* (2005) was carried out by projecting the spindle orientation onto a 2D-plane. She noted that this is statistically incorrect for the description of 3D-relationships and that these require sophisticated mathematical methods. Maro replied that the 2D-projection produced no bias and is mathematically correct as far as the angular relationship was concerned. Furthermore, according to Zernicka-Goetz, the pattern of the second cleavage and the identity of the 4-cell blastomere is defined and classified, based on the position of each blastomere relative to the second polar body after the second cleavage (Piotrowska-Nitsche and Zernicka-Goetz, 2005), in accordance with Gardner (Gardner, 2002). However, Louvet-Vallee noted that, in addition to the fact that the two second cleavages were randomly oriented, the forming 4-cell blastomeres dynamically change their relative position during and after cleavage (Louvet-Vallee *et al.,* 2005).

k. Contribution of the 4-cell blastomere (ME) descendants to the blastocyst

Zernicka-Goetz (Piotrowska-Nitsche and Zernicka-Goetz, 2005) reported the strong tendency of early-dividing two-cell blastomeres to take on the embryonic-fate only when they underwent meridional cleavages and the later-dividing ones had equatorial cleavages. This specific sequence of second cleavages, designated as the ME-type, was observed in about 40% of the examined embryos.

The orientation of the two second cleavages was not attended to in the studies that claimed no relationship between cleavage timing and embryonic-abembryonic polarity (Gardner, 2001; Fujimori *et al.,* 2003; Motosugi *et al.,* 2005; Alarcon and Marikawa, 2005), raising the question whether the embryos used in those studies exhibited the ME-type of second cleavages. Although further investigations are necessary, the classification of M and E cleavages may be practically impossible because the location of the second polar body, which is not stably associated with the animal pole in many cases (see ***b***), is used to define the orientation of second cleavages. In addition, the developmental potency of chimeric mice composed solely of the "specific" blastomere (ME) of the 4-cell embryo, reported to be defective (Piotrowska-Nitsche *et al.,* 2005), remains to be re-examined by others. This may nevertheless also be tampered by the difficulty in classifying ME blastomeres.

I. How does blastocyst cavity formation initiate?

The eventual position of the blastocyst cavity specifies the first embryonic polarity, the embryonic-abembryonic axis. Cavity formation initiates from a single point according to Zernicka-Goetz's lab, while Hiiragi, Fujimori and Maro (Hyenne *et al.,* 2005) commonly observe that the blastocyst cavity is formed initially as multiple cavities. This also needs to be reassessed by other groups: the initial cavity formation can be observed in the embryo at 85-100 hours post hCG injection (Motosugi *et al.,* 2005).

Future Directions

About two dozen active mouse researchers (see list of "Meeting Participants" on p. 581) literally from all over the world gathered in Freiburg in September last year, to discuss the current controversy regarding the patterning mechanisms of mouse pre-implantation embryos. This one-day meeting was apparently too short to resolve many of the issues, as summarized above. We apologize that this paper largely describes rather specific and technical aspects of the studies, which may be unfamiliar to non-mammalian researchers and perhaps even to many mammalian researchers. However, it is inevitable to describe a certain level of detail, because the source of the contradictions appears to stem from the differences in such details, e.g., experimental conditions, definitions of terms and interpretation of data. If we were to ignore these details, we would never be able to truly understand the mechanisms of mammalian development.

We wish this paper to fulfill the following two purposes: 1) to highlight the contentious matters and to reinforce the idea, particularly among scientists who are not directly involved in this field, that current knowledge of the precise mode of mouse early development is still unsettled and 2) to help those researchers who are already working on mouse early embryos and also those who are about to start working on this subject, to recognize what really are the problems and what needs to be done.

KEY WORDS: *mammalian preimplantation development, determinant, prepatterning, blastocyst axes, regulative model*

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Responses

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Unfair debate

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Hiiragi and colleagues have spent a great deal of energy in generating this artifical debate. They have chosen to do this by continually mis-representing papers from both Zernicka-Goetz' and Gardner's groups. For Hiiragi and colleagues to equate determination with the "pre-patterning" model for the development of the mouse embryo and state that it argues against regulative development is an extraordinary interpretation of the side that they contrive to oppose. Zernicka-Goetz and Gardner have never said that mouse development is determined, but quite the opposite. However, both these groups found bias (in other words pattern) in early development. Thus the mouse embryo does not develop entirely at random as previously assumed to be the case. The patterning model is a regulative model as bias does not connote determination but an inclination (see Zernicka-Goetz, 2006). It is seriously misleading to the community to: 1. criticise someone for something that has not been said and 2. to state patterning does not allow regulation - it does, in mouse as well as in other animals.

So far this debate seems to have generated more heat than light. It is a fire that has been enthusiastically stoked by Hiiragi and Solter. They erected an articifial foundation for the debate, have written and spoken extensively about it, orchestrated this meeting in their own Institute, and now publish a report that presents only one side (their own). It was unfortunate that Richard Gardner was not present at the meeting to support the view that there are chinks in old dogmas. There is an important body of older experimentation on the pre-implantation embryo that has not been undermined by the new findings. In fact, the discoveries of Zernicka-Goetz' and Gardner's labs do not run counter to this old body of data but rather to their rigid interpretation. It is therefore particularly disappointing that the authors of this report seem not to have gained any further appreciation of some of the concepts being put forward by the other side.

New concepts always meet difficulties in coming to sit alongside comfortable familiar ways of looking at the world. It takes some bravery to be able to present them. There will always be criticism from some gurus in comfortable positions who established the status quo and try to protect it. However, if as scientists, we are convinced of our new findings and if different approaches all lead to the same conclusion, then it is our duty to report them.

ZERNICKA-GOETZ M. (2006). The first cell-fate decisions in the mouse embryo: destiny is a matter of both chance and choice. *Curr Opin Genet Dev.* 16:406-12.

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Science is not a democracy

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> *"La parole ne représente parfois qu'une manière, plus adroite que le silence, de se taire"#*
> Simone de Beauvoir (1908-1986)

Science is not a democracy. Even if a huge majority of the meeting participants did not agree with the results or interpretations of the Zernicka-Goetz lab, the definitive answer as to whether there is any hereditary bias in mouse development needs hard data obtained independently in different laboratories. Before such hard data is obtained, we can only discuss the relevant issues, as we did in Freiburg. For that reason it seems premature to state that such a hereditary bias really exists in mouse early embryo, as stated by recent publications (e.g. Lawrence and Levine, 2006) and numerous text-books all over the world. It is puzzling that the hypotheses of the Zernicka-Goetz and Gardner laboratories have been so easily accepted by non-mammalian developmental biologists and that in contrast, these hypotheses are not so comfortably at home within the family of mouse embryologists (e.g. Hiiragi *et al.,* 2006). Nevertheless, the reason for this may be very simple: we cannot repeat many of the reported experiments and others have not tried.

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 # Translation: "A word is sometimes only the means, more appropriate than silence, to say no more."

Note from the Editor: Dr. Magdalena Zernicka-Goetz declined to respond to this *Developmental Perspective* paper, since she considered that an excessive number of inaccuracies would have to be corrected. She considers that her recent review in *Curr. Opin. Genet. Dev.* (2006), to which the reader is referred, provides her balanced view on current discrepancies.

ZERNICKA-GOETZ M. (2006). The first cell-fate decisions in the mouse embryo: destiny is a matter of both chance and choice. *Curr Opin Genet Dev.* 16:406-12.