temperature — Jones *et al.* find a three to four times greater warming by greenhouse gases. In the context of mitigation of climate change, a further consideration is that black carbon is usually co-emitted with other aerosols, such as sulphate, that cool the climate. Reductions in total aerosol output might be desirable for public-health purposes. In the context of climate change, however, appropriate technical means would have to be applied to reduce the warming influence of black carbon but not the (probably larger) cooling effect of other aerosols.

Finally, it is also necessary to remember that anthropogenic aerosols, including black carbon, have a very short atmospheric lifetime compared with that of greenhouse gases. The gases typically have lifetimes of centuries and longer[3], compared with days for aerosols. This implies that, if implementation of emission-reduction strategies were indeed to be feasible, climate-change mitigation by cutting black-carbon emissions could be effective fast. But it also suggests that the relative importance of black carbon will in any case gradually diminish, given that greenhouse gases are long-lived and that they will continue to accumulate in the atmosphere as long as anthropogenic emissions of these gases continue. ■

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DEVELOPMENTAL BIOLOGY

A mouse is not a cow

Early cell-lineage decisions during embryonic development differ between mice and cows. This finding calls for a re-examination of developmental variations across mammals, but does not undermine use of the mouse as a model organism.

JANET ROSSANT

The mammalian blastocyst is a thing of beauty. Over a period of a few days after the union of an egg with sperm, the fertilized egg divides to generate this tiny hollow sphere of cells, which has a cluster of enclosed cells at one end of the fluid-filled cavity. The outer cells are called the trophectoderm and the inner cells are, inventively, named the inner cell mass. But when do cells commit to becoming one or the other, and how? Writing in *Developmental Cell*, Berg *et al.*[1] show that the answers to these questions are not the same for mice and cattle.

Pluripotency — a cell's ability to differentiate into all cell types of the body — is a common property of the inner cell mass (ICM) of all mammalian blastocysts and is always associated with the expression and function of the transcription factor Oct4. The trophectoderm, which later generates all of the specialized layers of the placenta, also expresses a number of lineage-restricted transcription factors, most notably Cdx2.

In mice, deletion of either the *Oct4* gene (also known as *Pou5f1*) or the *Cdx2* gene leads to the formation of abnormal blastocysts: ICM cells of *Oct4*-mutant blastocysts express trophectoderm markers and lose pluripotency[2], whereas the outer cells of *Cdx2*-mutant blastocysts express pluripotency markers such as Oct4 ectopically and fail to differentiate further down the trophectoderm lineage[3]. This suggests a model — albeit an overly simplistic one — whereby restricted expression of Oct4 and Cdx2 leads to reciprocal repression of the opposing lineage and establishes cell fate.

Berg *et al.*[1] asked whether this model applies to cell-fate decisions in cows. They find that, unlike in mice, Oct4 expression is not restricted only to the ICM during the early stages of cow blastocyst development. Instead, Oct4 is co-expressed with Cdx2 in the trophectoderm for some time after the beginning of blastocyst formation. This observation is consistent with previous reports and has also been made for pig and human embryos (for example, see refs 4, 5). Even in the mouse, Oct4 expression overlaps with Cdx2 expression during the late cleavage and early blastocyst stages of embryonic development, and is restricted to the ICM only by the fully expanded blastocyst stage[3].

So why is Oct4 expression maintained for longer in the cow trophectoderm than in its mouse equivalent? Through experiments involving cow blastocysts engineered to express a fluorescently tagged version of mouse *Oct4* (the mouse *Oct4–GFP* transgene), Berg and co-workers show that the factors that restrict Oct4 expression to the ICM are not available, or not functional, in the cow blastocyst (Fig. 1a). Cdx2 could be one such factor, but the authors' data suggest that this protein has a role only later during cow embryonic development. However, Berg and colleagues do not investigate whether the role of Cdx2 in restricting Oct4 expression is simply delayed in the cow embryo, nor whether Oct4 is ectopically expressed later during development in embryos treated to express reduced levels of Cdx2.

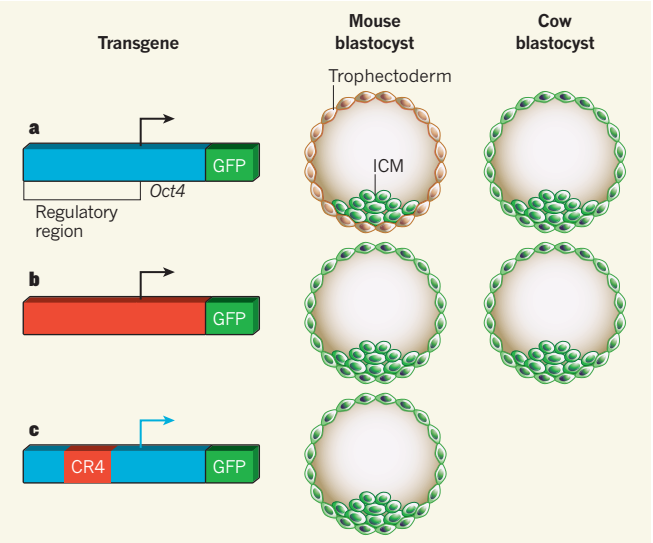

Figure 1 | Oct4 regulation in mouse and cow blastocysts. **a**, Berg *et al.*[1] find that the expression of a *GFP* fluorescent reporter transgene controlled by the regulatory elements of the mouse *Oct4* gene (blue) is restricted to the inner cell mass (ICM) in mouse blastocysts but not cow blastocysts. **b**, The same transgene, but containing the bovine Oct4 regulatory elements (red), is not restricted to the ICM in either cow or mouse blastocysts. **c**, The authors narrow down this effect of bovine regulatory elements to the CR4 region.

The paper[1] shows that a mouse *Oct4–GFP* transgene containing the bovine Oct4 regulatory elements is expressed in both the ICM and trophectoderm in fully expanded blastocysts of both the cow and the mouse (Fig. 1b). This suggests that Cdx2, which is active in mouse blastocysts, is not the only factor that affects the timing of Oct4 repression. It also indicates that bovine regulatory elements do not respond to the factors that downregulate Oct4 in mouse blastocysts.

Of the four evolutionarily conserved regulatory regions around the Oct4 locus, CR4 shows the most sequence divergence between the mouse and the cow. When Berg *et al.* replaced mouse CR4 with the cow version in the mouse *Oct4–GFP* construct, it behaved like the cow gene in the mouse blastocysts (Fig. 1c). Thus changes in both DNA

regulatory regions and the factors that bind to such sequences drive differences in the regulation of Oct4 expression between mouse and cow blastocysts.

It would be interesting to test, in transgenic mice, whether regulatory elements of the human *OCT4* gene behave like the mouse or the cow sequences. Although human blastocysts, like those of domestic animals, express Oct4 in the trophectoderm for an extended period compared with mice, the period of overlap of Cdx2 and Oct4 expression is only slightly longer than in the mouse. Human OCT4 is clearly restricted to the ICM by day 6 before embryo implantation[6].

But why do these regulatory differences exist among the blastocysts of different mammals? Evolutionarily, the placenta is a recent invention, and still seems to be a work in progress. There is huge variation in trophectoderm and placental morphology across different mammalian species, accompanied by recent evolutionary divergence in placenta-specific gene families[7]. For example, a mouse blastocyst attaches and implants in the uterus by embryonic day 5 (E5); a human blastocyst grows a little larger but then implants by E7–9 with highly invasive trophoblast outgrowth; and in cows, pigs and sheep the blastocyst floats in the uterus for 2–3 weeks before attaching.

Berg *et al.* propose that such differences lead to earlier restriction of trophectoderm cell fate in the mouse than in the cow. Indeed, results of their experiments — involving chimaeric blastocysts generated by mixing trophectoderm cells from different stages of development with host embryos — support this proposal.

In a remarkable technical tour de force, they also transferred the chimaeric cow blastocysts to recipient cows and recovered them later in development to show that early trophectoderm cells can contribute to developing ICM derivatives. This is one of the first attempts to test the timing of lineage restriction in a species other than the mouse.

This study emphasizes the need to explore the timing and mechanism of functional lineage restriction in blastocysts of different mammals, including humans. Differences in these parameters may underlie the known difficulty in deriving validated pluripotent embryonic stem cells and trophoblast stem cells from many mammalian species. Although fibroblasts have been reprogrammed into induced pluripotent stem cells in several domestic species, including the cow, these lines often depend on continued expression of exogenous reprogramming factors. Clearly, we need a better understanding of the control of pluripotency in all these species.

As we learn more about the precise details of mouse blastocyst development, we must be constantly evaluating similarities and differences between them and those of humans and other species. This will help us to truly understand mammalian embryo diversity. ■

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MOLECULAR BIOLOGY

A fly in the face of genomics

The modENCODE project uses integrative analysis to annotate genomic elements in the fruitfly and a nematode worm. The first fly data have now been published. **SEE ARTICLES P.473 & P.480 & LETTER P.527**

EILEEN E. M. FURLONG

The fruitfly *Drosophila melanogaster* is an exceptional model for dissecting the basic principles of biology, development and disease. It is amenable to genetic manipulation using tools developed over more than a century; and its genome shares extensive genetic content with humans. The first draft of the *Drosophila* genome was released a decade ago[1], and with subsequent updates its annotation is in a 'mature' state. Nevertheless, more than half of the predicted genes have been awaiting experimental verification of their structure — the location of promoter sequences, of boundaries of protein-coding and non-coding sequences, and of transcription termini. The modENCODE consortium project aims to address this issue and to identify new genes and genomic elements in the fly genome[2]. Here I focus on the first wave of papers, including three in this issue[3–5], which describes the fly data so far.

To determine which genes are expressed at specific stages of development, Graveley *et al.*[3] (page 473) generated high-resolution expression data, which are complemented by an analysis of 25 *Drosophila* cell lines[6,7]. These efforts identified almost 2,000 new genes that encode proteins or non-coding RNAs. They also extensively refine existing annotation by describing more than 3,000 new promoter sequences[7], roughly 53,000 new or revised exon sequences[3], a threefold increase in RNA-splicing events[3] and a tenfold increase in RNA-editing events[3]. Notably, most of the RNA-editing and -splicing events occur at precise stages of the *Drosophila* life cycle, indicating extensive temporal regulation of these post-transcriptional events by as-yet poorly understood mechanisms. This comprehensive view of the fly transcriptome[3,6,7] reveals that some 75% of the organism's genome is transcribed at one stage or another — in line with the widespread transcription observed in other species.

Post-translational histone modifications covering a gene's promoter or coding region provide telltale signatures of the expression status of a gene and thereby present another way to identify functional elements in the genome. Two of the modENCODE studies involved mapping such chromatin marks in *Drosophila* cell lines[4] and at 11 stages of its life cycle[5].

By examining the distribution of 18 histone modifications in two cell lines, Kharchenko *et al.*[4] (page 480) identified nine prominent chromatin signatures, which complement those defined previously[8]. Clues to their function come from information on chromatin accessibility and transcriptional activity, revealing chromatin signatures that distinguish between active and inactive genes, active promoters, and the location of new putative regulatory elements. The authors' global analyses[4] extend previous studies[9–12] indicating that the Polycomb system — a group of chromatin-binding proteins traditionally associated with stable, long-term gene repression during embryonic development — can also function dynamically and associate with promoters that are actively transcribed or seem poised for activation.

Deposition of chromatin marks is linked to the enzymatic activity of RNA polymerase during the initiation and elongation steps of transcription; this activity is regulated by transcription factors bound to *cis*-regulatory elements — proximal and distal sequences that affect gene expression. To understand how transcription is regulated, Nègre *et al.*[5] (page 527) made a systematic effort to identify all *cis*-regulatory elements by examining the occupancy of 38 transcription factors and other chromatin-regulatory proteins at different stages of development. The result is a collection of around 20,000 putative regulatory elements that include insulators, enhancers and