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Distributional Changes in Lysosome-Like Bodies in Mammalian Embryos during the Course of Blastocyst Formation

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Abstract: *Distributional changes in lysosome-like bodies (LBs) in mouse, rat, rabbit and bovine embryos during the course of blastocyst formation were studied with acridine orange (AO) staining. AO-stained LBs were distributed throughout the cytoplasm of all round blastomeres in untransformed 8- and 16-cell mouse embryos, 32-cell rabbit embryos and 16-cell bovine embryos, whereas they were observed from the nucleus to the apical cytoplasm of all round blastomeres in 8-cell rat embryos. In morulae of these animals, LBs were present in the entire cytoplasm of inner round blastomeres, and were localized around the nucleus in outer flattened (mouse) and cuboidal (rabbit and cow) blastomeres or the apical cytoplasm in outer flattened (rat) blastomeres.In blastocysts, LBs were present in the entire cytoplasm of inner-cell-mass cells and around the nucleus of trophoblast cells. Such distribution of LBs in mouse embryos was also observed in those developed in vitro. From these results, it was suggested that there is a close relationship between distributional changes in LBs and the transformation and differentiation of blastomeres in mouse, rabbit and bovine embryos.*

Key words: *Mammalian embryo, Blastocyst formation, Lysosome-like body, Acridine orange.*

Based on electron microscopic observation [1–5], fluorescent microscopic observation of acridine orange (AO)-stained specimens [6–9] and immunohistochemical observation with anti-lysosome-like body (LB) antibody [10, 11], it is confirmed that mammalian embryos of many species contain LBs during early development, but reports on distributional changes in LBs during the course of blastocyst formation have been published as rarely as those only on the mouse [5, 8] and golden hamster [9]. It was revealed that LBs were distributed throughout the cytoplasm of blastomeres in untransformed 8- and 16-cell mouse embryos, and were observed in the apical cytoplasm of flattened blastomeres when blastomeres began to show signs of transformation, and around the nuclei of trophoblast cells in blastocysts. From these results, Batten *et al.* [8] considered that the distribution of LBs could not be used as an accurate marker for the transformation of flattened blastomeres to trophoblast cells. On the other hand, we postulated that distributional changes in LBs were closely involved in differentiation of blastomeres because the distribution of LBs in flattened blastomeres was comparable to that seen in trophoblast cells [9].

Distributional changes in LBs in mammalian embryos during the course of blastocyst formation have been investigated only in the mouse and golden hamster but not in any other animals. In the present study, we observed distribution of LBs in mouse, rat, rabbit and bovine embryos during the course of blastocyst formation to determine whether distributional changes in LBs are involved in the differentiation of blastomeres. In addition, we also observed distributional changes in LBs in mouse embryos developed *in vitro* to compare them with embryos developed *in vivo*.

Materials and Methods

Animals

Forty female mature mice of the ICR strain, 24 female mature rats of the Wistar strain, 11 mature female Japanese White rabbits and 18 mature Japanese Black and Holstein cows were used. The mice, rats and rabbits were kept and fed normally in a room at 24°C and lit 14 h a day, from 4 a.m. to 6 p.m. Bovine ovaries were obtained from a slaughterhouse.

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Embryo collections

Mouse: Embryos were obtained by superovulation: the animals were subjected to peritoneal injections with 5 i.u. of PMSG (Serotropin, Teikoku Hormone Manufacturing Co., Japan) and with 5 i.u. of hCG (Gonatropin, Teikoku Hormone Manufacturing Co.) 48 h later, and then they were mated with ICR males of proven fertility. Embryos at the untransformed 8- and 16-cell, morula and blastocyst stages were recovered from oviducts or uteri of 20 females 67, 80 and 96 h after hCG injection.

To obtaine embryos developed *in vitro*, 2-cell embryos were recovered 48 h after hCG injection from oviducts of superovulated and mated 20 mice. These embryos were cultured in M16 medium [12] at 37°C in a CO_2 incubator (5% CO_2, 95% air), and the embryos at the untransformed 8- and 16-cell, morula and blastocyst stages were obtained after 20, 32 and 52 h of culture.

Rat and Rabbit: Embryos were obtained by spontaneous ovulation, that is, rats were mated with fertile males at estrous, and the next morning the presence of sperm was ascertained in the vaginal smear (Day 1). Embryos were recovered from oviducts or uteri at 9 a.m. on Day 4, 8 p.m. on Day 4 and 5 p.m. on Day 5, so that they were at the untransformed 8-cell, morula and blastocyst stages. Rabbit embryos at the untransformed 32-cell, morula and blastocyst stages were recovered from oviducts or uteri of mated females at 55, 85 and 95 h after mating.

Cow: As in our previous report [13], immature eggs were collected by sucking out of visible follicles and cultured for maturation, and cumulus-egg complexes after maturation culture were inseminated with sperm of the same species. After insemination, cumulus-egg complexes were cultured at 39°C in TCM-199 containing 5% FCS (Gibco Lab., USA). Embryos used were at the untransformed 16-cell stage (4 days after insemination), the morula stage (5 days) and the blastocyst stage (7 days).

Acridine orange staining

In order to demonstrate LBs, embryos from mice, rats, rabbits and cows were washed 3 times in Earle's balanced salt solution (pH 7.3) [14] containing 25 mM HEPES (Wako Pure Chemical Industries, Ltd., Japan) and 0.3% bovine serum albumin (Sigma Chemical Co., USA) (mEBSS), and then immersed in mEBSS containing 0.2% pronase (Sigma Chemical Co.) to dissolve the zona pellucida. These embryos were immersed in a staining solution composed of 500 μg AO (Sigma Chemical Co.) and 100 ml mEBSS for 5 min at room temperature. After staining, the embryos were washed 3 times in mEBSS and placed on glass slides to be photographed under a reflected-light fluorescing microscope (Nikon Corporation, Japan). The same procedures for the demonstration of LBs were applied 3 times to 15 to 25 embryos from each developmental stage.

Results

Morphology of embryos

The blastomeres of untransformed embryos of all animals were spherical, but in the morulae, outer blastomeres were flattened in the mouse and rat, and cuboidal in the rabbit and cow. The morulae of mice and rats had compacted, while those of rabbits and cows had not. Blastocysts were composed of spherical inner-cell-mass cells and flattened (mouse and rat) or cuboidal (rabbit and cow) trophoblast cells.

Distribution of LBs

Untransformed embryos: LBs were distributed throughout the cytoplasm of all round blastomeres in mouse, rabbit and bovine embryos before blastomeres were transformed (Fig. 1a), whereas LBs were aggregated in the columnar form in the apical cytoplasm above the nucleus of all round blastomeres in rat embryos (Fig. 1b).

Morulae: In morulae of mouse, rabbit and cow, LBs were distributed over the entire cytoplasm of inner round blastomeres, and were observed around the nucleus, particularly in the region between the basolateral membrane and nucleus, in outer flattened (mouse, Fig. 1c) or cuboidal (rabbit and cow) blastomeres. In rat morulae, LBs were distributed throughout the cytoplasm of inner round blastomeres and were aggregated in the columnar form in the apical cytoplasm above the nucleus of outer flattened blastomeres (Fig. 1d).

In all animals examined, a very few morulae showed different distributional patterns of LBs from those described above. Namely, LBs were observed in the entire or apical cytoplasm of flattened or cuboidal blastomeres.

Blastocysts: In blastocysts of all the animals, LBs were distributed around the nucleus of trophoblast cells and over the entire cytoplasm of inner-cell-mass cells, showing no differences among animal species (Fig. 1e–h).

Mouse embryos developed in vitro: Distributional changes in LBs were the same as those observed in embryos developed *in vivo*, as mentioned above.

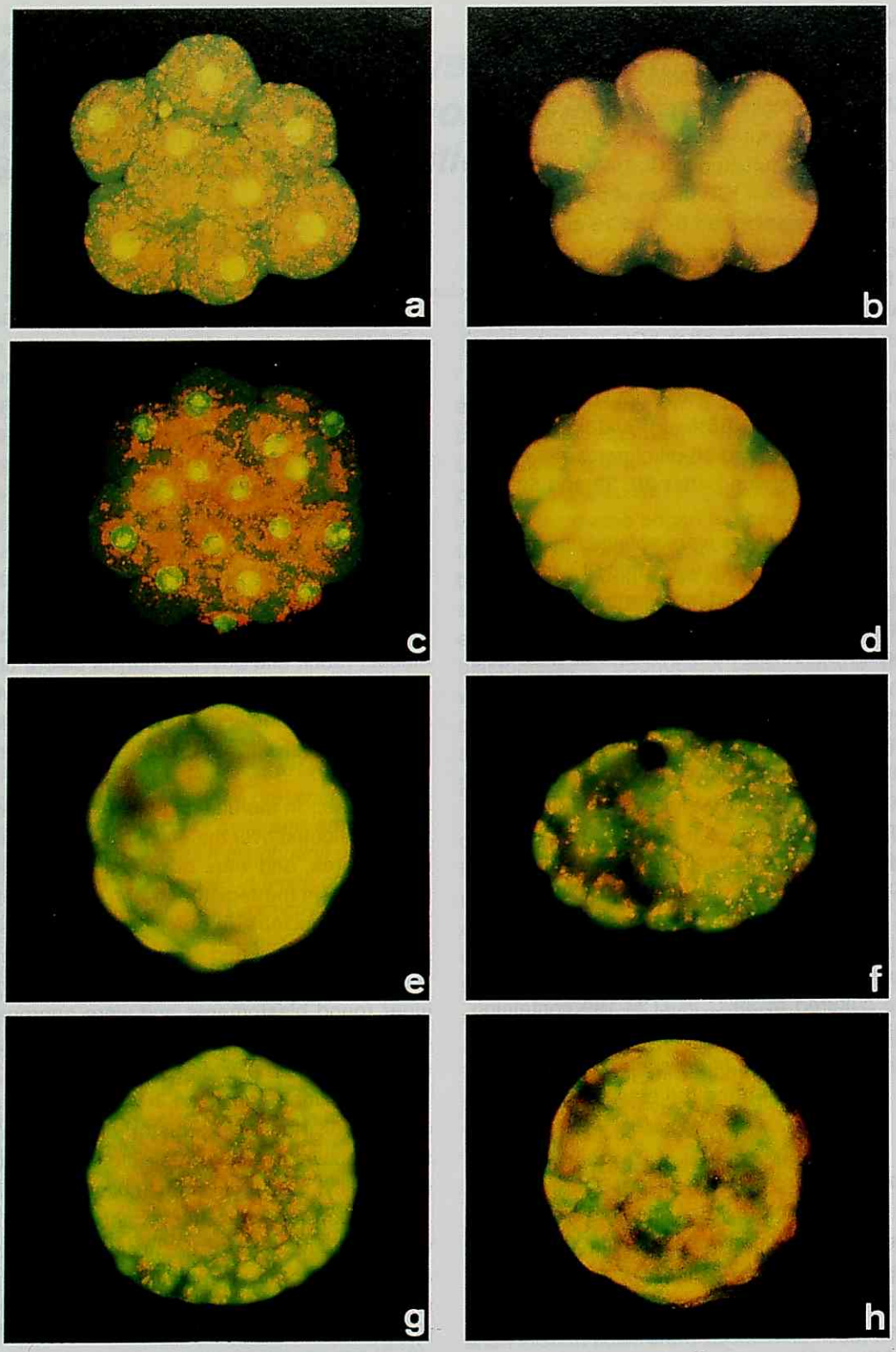

Fig. 1. Fluorescent microphotographs of embryos after staining with acridine orange. Orange-yellow fluorescent granules showing the presence of lysosome-like bodies are seen in the cytoplasm. (a) Untransformed 8-cell mouse embryo. × 400. (b) Untransformed 8-cell rat embryo. × 400. (c) Mouse morula. × 400. (d) Rat morula. × 400. (e) Mouse blastocyst. × 400. (f) Rat blastocyst. × 400. (g) Rabbit blastocyst. × 200. (h) Bovine blastocyst. × 200.

Discussion

When LBs were demonstrated in mouse, rat, rabbit and bovine embryos during the course of blastocyst formation, their distribution was found to alter at the time of transformation of blastomeres in all animals examined, but not in the rat, suggesting the involvement of distributional changes in LBs in the transformation of blastomeres. It was therefore inferred that transformation of blastomeres occurred due to redistribution of cytoplasmic organelles, including LBs, as suggested by Reeve [7] and Batten *et al.* [8].

As confirmed by our previous experiments in golden hamster embryos [9], it was also ascertained in the present study that distribution of LBs in transformed blastomeres was comparable to that in trophoblast cells of blastocysts in mice, rabbits and cows. Thus it was assumed that transformed blastomeres of morula were translated into trophoblast cells of blastocysts in the golden hamster, mouse, rabbit and cow. It was also determined in mice that distributional changes in LBs occurred in embryos developed *in vitro* similar to those developed *in vivo*. On the other hand, it was revealed that distribution of LBs in rat embryos showed no clear changes at the time of transformation of blastomeres, and distribution of LBs in transformed blastomeres differed from that in trophoblast cells of blastocysts, disclosing different distributional changes in LBs in the rat from those in other animal species examined. Further studies are needed to determine whether distribution of LBs seen in transformed blastomeres of rat embryos is characteristic of the rat, or rat embryos require a longer time than embryos of other animals for redistribution of LBs in association with transformation of blastomeres.

Fleming and Pickering [5] and Batten *et al.* [8], who observed distribution of LBs in mouse embryos, stated that LBs were distributed over the apical cytoplasm of transformed blastomeres, differing from the results obtained with mouse embryos in the present study. The precise reason for this discrepancy is unclear. On the other hand, Maro *et al.* [10], who immunohistochemically investigated distribution of LBs in transformed blastomeres of mouse morulae by using anti-LB antibody, reported similar distribution of LBs to that observed in the present series of experiments.

It is generally accepted that LBs are cytoplasmic organelles playing a role in intracellular digestion of foreign bodies of intra- and extra-cellular origin. It is said that the ability to uptake extracellular proteins increases in 8-cell rat embryos [15] and in the stage from morula to blastocyst of rabbit embryos [16], and that the activity of acid phosphatase, a marker enzyme of LBs, appears in 8-cell embryos of the mouse [17–19] and golden hamster [20] and is enhanced in blastocysts. It is therefore inferred that LBs present in mammalian embryos during the course of blastocyst formation not only show distributional changes, but also play an active role in digestion of exogenous proteins.

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