Cerebral Cortical or Cerebellar Nuclear Lesion-induced Synaptic Reorganization in the Basilar Pons of the Rat

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In the rat basilar pons, synaptic reorganization of the cerebellopontine (or corticopontine) terminals in response to neonatal cortical (or cerebellar nuclear) lesion is as follows: In response to neonatal cortical lesion, cerebellopontine fibers sprouted to occupy distal dendritic locations along pontine neurons. Following cerebellar nuclear lesions, corticopontine fibers sprouted to occupy proximal dendritic locations or made glomerular synaptic complexes with several dendritic appendages. Above results correlate well with the light microscopic study which indicated increased terminal density in the basilar pons following cortical (or cerebellar) lesions and provide a neuroanatomical evidence for gradual recovery of cerebellar functions following neonatal cerebellar nuclear lesions.

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Production of Monozygotic Multiplets from 8-cell Mouse Embryos through the Construction of Chimeric Embryos

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To obtain monozygotic multiplets from 8-cell mouse embryos, we artificially constructed chimeric embryos by introducing one blastomere (donor) of 8-cell embryos of F1 hybrid (C57BL/6 × CBA) mice into 4-cell ICR mouse embryos (carrier) of which one blastomere had been previously removed with a micromanipulator. After 42 h of culture, the developmental frequency of chimeric embryos to normal morula and blastocyst was 95% (310/328). When chimeric embryos at morula or blastocyst stage were transferred to pseudopregnant mice, 39% (70/180) of them were born. Most of the offspring (56/70) were the carrier type in coat color, whereas only three of them were the donor type, of which two were assumed to be derived from single 8-cell donor embryo. Because the two donor type mice were the same sex and produced only the donor type offspring from a testcross, they are probably monozygotic multiplets of 8-cell mouse embryos. However, since their internal chimerism was not able to be examined, it remains to be determined if their genetic constitutions are identical.

KEY WORDS: Monozygotic multiplets, Chimeric embryos, Micromanipulation.

In preimplantation development of mouse embryos, the timing of blastomere divisions is asynchronous after 2-cell stage (Graham and Deussen, 1978; Graham and Lehtonen, 1979). This asynchrony of cleavage leads to some blastomeres that are smaller and more advanced than other blastomeres of the embryo. These smaller, more advanced blastomeres tend to migrate to inner part of morula and inner cell mass of blastocyst (Kelly *et al.*, 1978; Spindle, 1982; Surani and Barton, 1984). Therefore, in the chimeric embryos that are constructed with asynchronous blastomeres, the inner cell mass of chimeric blastocyst and their live young will be able to originate from more advanced blastomeres (Seidel, Jr., 1983; Willadsen and Fehilly, 1983). Actually, Willadsen and Fehilly (1983) obtained monozygotic quintets from 8-cell sheep embryos by aggregating one blastomere of 8-cell embryos with one of 4-cell blastomeres. We conducted experiments to determine if chimeric embryos constructed with blastomeres at different cell stages in mouse embryos were capable of developing into monozygotic multiplets.

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Materials and Methods

ICR and F1 hybrid (C57BL/6 × CBA) strain mice were supplied by Biopotency Evaluation Program of Genetic Engineering Research Institute, KIST. The female mice were superovulated by intraperitonial injection of 5 IU pregnant mare's serum gonadotropin (Teikokuzoki, Japan), followed by the injection of 5 IU human chorionic gonadotropin (Sigma, U.S.A.) 48 hours later. They were mated with male mice of the same strain.

Successful matings were verified by the presence of copulatory plug in the following morning.

Embryos were cultured in M16 media (Whittingham, 1971) supplemented with 4 mg/ml bovine serum albumin (BSA) in a humidified atmosphere of 5% CO_2 in air at 37°C. Micromanipulation was carried out on an inverted Nikon Diaphot microscope (Japan) with a Leitz micromanipulator (W. Germany) in M2 media (Quinn *et al.*, 1982) supplemented with 4 mg/ml BSA at about 30°C.

Preparation of 8-cell and 4-cell embryos

In this experiment, we designated single blastomeres of 8-cell embryos as donors and 4-cell embryos of which one blastomere had been previously removed as carriers, respectively. The experimental groups were divided into compact and uncompact chimeric embryos according to the degree of compaction of donor 8-cell embryos. Donor 8-cell embryos were obtained from homozygously pigmented F1 hybrid mice. To obtain uncompact 8-cell donors, 1-cell embryos were recovered at 18-20 h post-hCG injection, freed of their cumulus cells by a brief exposure to 1 mg/ml hyaluronidase (Sigma, U.S.A.) and cultured for 52 h. For the preparation of compact 8-cell donors, 2-cell embryos were recovered by flushing from the oviduct at 44-46 h post-hCG injection and cultured for 22 h. Carrier 4-cell embryos were prepared by oviduct flusing from albino ICR mice 52-54 h after hCG injection.

Construction of chimeric embryos

For the production of chimeric embryos from donors and carriers, a part of zona pellucida of all embryos were cut and at the same time one blastomere of each 4-cell carrier embryo was destructed with a sharp micropipet. After 20 min of preconditioning in Ca^{2+}-free media, individual blastomeres were dissociated from donor 8-cell embryos with a narrow bore micropipet and then introduced one by one to the inside of zona pellucida of each carrier embryo. So, we were able to make a set of 8 chimeric embryos from one 8-cell donor embryo and eight 4-cell carrier embryos (Lee and Lee, 1990).

Developmental potency test of chimeric embryos and their phenotype expression

Each set of chimeric embryos was cultured for 42 h, and normally developed morula and blastocyst embryos were transferred to one of uterine horns of pseudopregnant mice on the third day of pregnancy to test their developmental potency. Finally, the genotype of their offspring was identified by their coat colors and a testcross with albino ICR mice.

Results and Discussion

***In vitro* development of chimeric embryos**

When the chimeric embryos were cultured *in vitro* for 42 h (Table 1), most of them (310/328) developed to normal blastocyst and morula

Table 1. Preimplantation development of chimeric embryos constructed with one donor blastomere of 8-cell F1 hybrid embryo and 4-cell ICR carrier embryo.

	Donor	Carrier	No. of chimeric embryos	No. of embryos after 42 hrs of culture			
				Blastocyst:	Morula:	Abnormal:	Damaged
Experimental groups	Uncompact hybrid 8-Cell	ICR 4-Cell	88	80	1	2	5
	Compact hybrid 8-Cell	ICR 4-Cell	240	219	10	7	4
	Total		328	299	11	9	9
Control group	ICR 4-Cell		31	30	1		

irrespective of compactness of their 8-cell donor embryos. Some (9/328) were damaged during micromanipulation (damaged) and others (9/328) were induced abnormal compaction and blastocoel formation (abnormal). Notwithstanding that the developmental stages of donors and carriers were different, the developmental potency of chimeric embryos was not significantly different from that of control embryos, suggesting that they communicate with and regulated one another to synchronize their time of blastocoel formation (Stern and Wilson, 1972; Prather and First, 1986). But it seems that there is a limitation in their regulatory capacity because some chimeric embryos did not induce normal compaction and blastocoel formation.

Embryo transfer and phenotypic expression of chimeric embryos

When normally developed chimeric embryos at morula and blastocyst stage were transferred to pseudopregnant mice (Table 2), 49% (70/180) of them were born to offspring simillar to the case of control transfer. When the offspring were classified according to their coat color, the majority (56/70) were carrier type and some (11/70) chimera type. But donor type mice of interest were only three in compact donor group. Even if the chimeric embryos did not predominantly developed to donor type mice, in one case (Table 3) of which 6 offsprings were developed from one set of chimeric embryos, two of them were donor type of the same sex. Moreover, they produced only donor type offspring

Table 2. Postimplantation development of chimeric embryos transferred to the uterus of day 3 pseudopregnant mice at blastocyst and morula stage.

	Donor blastomere of chimeric embryos	No. of embryo transferred	No. of off-spring (% of transferred)	Coat color type		
				Donor:	Chimera:	Carrier
Experimental groups	Uncompact 8-Cell	48	23(48%)	0	4	19
	Compact 8-Cell	132	47(36%)	3	7	37
	Total	180	70(39%)	3	11	56
Control group	ICR 4-Cell	29	12(41%)			

Table 3. Testcross records of mice produced from one set of 8-cell chimeric embryos.

Mouse	C1-1	C1-2	C1-3	C1-4	C1-5	C1-6
Sex	♀	♀	♀	♂	♂	♀
Coat color (% black)	100	100	50	95	50	0
No. offspring from testcross (No. litters)	27 (3)	18 (2)	37 (3)	29 (3)	33 (3)	31 (3)
Coat color of offspring						
black	27	18	14			
albino			23	29	33	31

when they were put into a testcross with albino ICR mice.

Meanwhile, generally accepted rules on the sex differentiation of chimeric mice are that most chimeras of mixed chromosomal sex (XX/XY) are to be phenotypic male with a rare exception and their germ cells are derived from only XY components. However, single chromosomal sex (XY/XY, XX/XX) chimeras are to be either male or female, respectively and their germ cells are to originate from each two components, so that the testcross results in mixed progeny production (McLaren, 1975; Evans *et al.*, 1977; Gearhart and Oster-Granite, 1981; Iannaccone *et al.*, 1985). Therefore, two donor type mice mentioned above seem to be genetically identical, because they were both females and produced only donor type offspring from a testcross. But, since their internal organ chimerism was not able to be determined, we are now carrying out the similar experiment with mouse embryos carring specific genetic markers.

Donor type mice were produced only in the compact group (Table 3). This suggests that 8-cell blastomeres of compact embryos are different in developmental potency from those of uncompact embryos. Even though a large number of donor type offspring were not developed from chimeric embryos, this experiment presents the possibility of producing monozygotic multiplets from 8-cell mouse embryos. The possibility is also supported by the results of Tsunoda *et al.* (1987) who obtained monozygotic triplets from 8-cell mouse embryos by using parthenogenetic embryos as carriers, although they could not determine the internal organ chimerism of the monozygotic triplets.

In the future, if specific genetic markers are used for determining the internal organ chimerism of monozygotic multiplets, we will be able to confirm their genetic constitution. In addition, we will be able to promote the efficiency of monozygotic multiplet production from 8-cell mouse embryos by changing the developmental stage and cell number of donors and carriers in the construction of chimeric embryos.

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Chimeric embryo의 구성을 통한 8세포기 생쥐 수정란으로부터의 일란성 다쌍자 생산

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생쥐 8세포기 수정란으로부터 일란성 다쌍자를 얻기 위하여, 교잡종(C57BL/6 × CBA) 생쥐 수정란의 8세포기 할구(donor)를, 하나의 할구를 없앤 4세포기 ICR 생쥐 수정란(carrier)에 하나씩 도입한 chimeric embryo를 미세조작기법으로 만들었다. Chimeric embryo중 정상적인 배반포기배 및 상실배로의 체외 발달율은 95%(310/328)였으며, 이들 중 정상적인 상실배 및 배반포기배로 발달한 것을 가친에 이식한 경우에는, 이식한 것 중에서 39%(70/180)가 산자로 태어났다. 이들 산자를 coat color에 따라 분류했을 때, 대부분(56/70) carrier type이었으며, donor type은 3마리가 태어났을 뿐이었다. 그러나 3마리의 donor type 산자 중 2마리는 하나의 8세포기 수정란에서 유래한 것으로써, 둘다 암컷일 뿐 아니라 이들을 검정교배하였을 때, 모두 donor type의 자손만을 생산하는 것으로 보아 이들 두마리는 유전적 동질개체인 것으로 판단된다.