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Original Article

Restriction of the fate of early migrating trunk neural crest in gangliogenesis of avian embryos

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ABSTRACT **Trunk neural crest is the source of peripheral nervous tissue, the adrenal medulla and pigment cells. To quantitatively assay the potency of neural crest to form each derivative tissue, we isolated fragments of neural crest from quail embryos and transplanted them into the migration pathways of chicken embryos. In the resultant chimeras, we counted the quail cells derived from grafts distributed in the dorsal root ganglia, the sympathetic tissues around the aorta and the spinal nerves. Descendant cells of quail neural crest derived from the brachial or lumbosacral and lower levels were more abundant in the dorsal root ganglia than in the sympathetic tissue, while those derived from adreno-medullary levels were more abundant in the sympathetic tissue than in the dorsal root ganglia. No correlation was seen between the distribution pattern of quail cells and the rostrocaudal levels of graft site in chick embryos. These findings suggest that the developmental potency of truncal neural crest in gangliogenesis is restricted in the early phase of their migration and differs along the rostrocaudal axis, although it is not clear whether this restriction reflects determination of each crest cell. The size of the rudiments of the dorsal root ganglia in the normal embryo differed along the rostrocaudal axis, these differences being consistent with those in the fate of the neural crest at a given somite level.**

KEY WORDS: *trunk neural crest, development, quail-chick chimera, dorsal root ganglion, sympathetic trunk*

Introduction

Neural crest cells appear transiently on the dorsolateral surface of the neural tube in early embryogenesis and subsequently migrate along specific pathways (Hall and Hörstadius, 1988; Hörstadius, 1950; Weston, 1963; Le Douarin, 1982; Loring and Erickson, 1987). After migration, they differentiate into various kinds of cells such as neurons including cholinergic and adrenergic cells in the peripheral nervous system, Schwann cells, pigment cells, adrenal medulla cells etc.

The neural crest gives rise to cells expressing a relatively limited set of phenotypes at a given level of the neuraxis in normal development (Hörstadius, 1950; Weston, 1963; Le Douarin, 1982). However, heterotopic transplantation experiments have shown that their fate could be changed. The vagal crest, which is the origin of cholinergic cells in enteric ganglia, differentiated into adrenergic cells when implanted at the level of somites 18-24. Conversely, the neural crest of the adrenomedullary level transplanted into the vagal area gave rise to enteric ganglia (Le Douarin and Teillet, 1973).

On the other hand, cranial and truncal crest do not possess the same potential for cytodifferentiation, at least quantitatively. When the cervicotruncal crest was transplanted into the cranial region, it produced only a small amount of cartilaginous and connective tissues, which are derivatives of cranial crest cells in normal development (Le Lièvre and Le Douarin, 1975; Nakamura and Le Lièvre, 1982). Thus, the fate of truncal neural crest appears to be interchangeable within the truncal levels, but can hardly change into cranial crest.

Another question regarding neural crest cell development is how the crest cells choose migration pathways and settlement sites. It is likely that determined crest cells choose their appropriate routes, as only the cells migrating along the dorsolateral pathway express the pigment cell precursor antigen (Kitamura *et al.*, 1992) The hypothesis that pre-determined cells choose their own pathway is inconsistent with their plasticity in cell differentiation. However, even the cholinergic ciliary ganglion in 4-15-day quail embryos produced adrenergic cells when back-transplanted into the neural crest migration pathway at the adrenomedullary level in younger chicken embryos (Dupin, 1984). This suggests that the cells undetermined in cell differentiation pursue a pathway to a certain ganglion.

The aim of the present study was to elucidate whether the trunk neural crest is determined to migrate and settle to produce dorsal root ganglia, sympathetic ganglia or adrenal medulla. We transplanted a small fragment of quail truncal neural crest into a neural crest migration pathway of chick embryos and pursued the transplanted cells until they settled to form dorsal root ganglia (DRG), sympathetic tissues around the aorta (ST), or spinal

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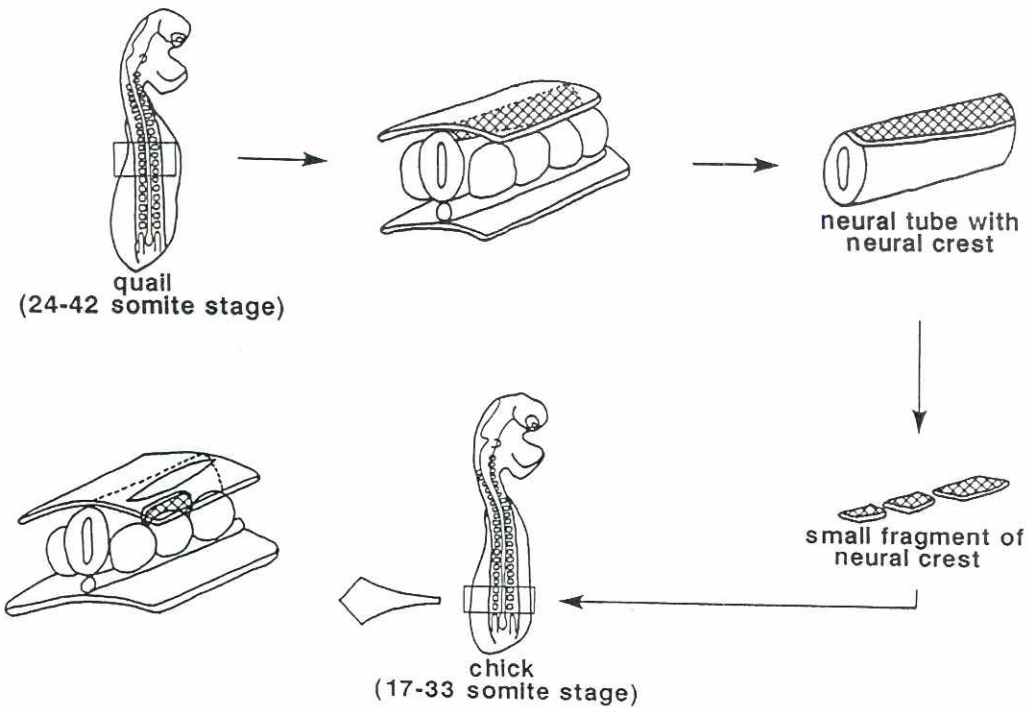

Fig. 1. Diagram of the operational procedure. *The tissue including the neural tube corresponding to 4 pairs of somites from caudal somites 10 to 7 was dissected out from 2.5- to 3-day quail embryos (at the 24-42 somite stage). The neural tube, together with the neural crest, was separated from the surrounding tissues, and a small fragment of neural crest was peeled off and transplanted into a slit made between the neural tube and the most caudal 3 somites of 2.5- to 3-day chick embryos (at the 17-33 somite stage). The chimeric embryos were further incubated for 2 days.*

nerve. The distribution patterns of quail cells in these tissues were assayed quantitatively and were shown to differ along the rostrocaudal level where the grafts were obtained, suggesting that the choice of migration pathway and possibly the cell growth of trunk neural crest may be determined in their early phase of migration.

Results

A piece of the neural crest at the level of the 7th to 10th somite from the most caudal somite was transplanted into a chick embryo between the neural tube and the most caudal two or three somites (Fig. 1). By this procedure, we obtained 66 chick-quail neural crest chimeras at stage 25 (Hamburger and Hamilton, 1951). All of these chimeras included descendants of the transplanted quail neural crest cells, which were distinguished from host chick cells by massed heterochromatin in the nuclei of quail cells in three tissues, i.e. the DRG, ST and spinal nerves, exclusively on the operated side (Figs. 2, 3). The numbers of quail cells found in each of the three tissues were counted, and then the ratios of the number of cells in each tissue to the total number of quail cells in each chimera were calculated (Table 1).

In this study, we counted quail cells only in these three tissues, because few quail cells were found in other neural crest derivatives, for example melanocytes, at stage 25. The total number of quail cells in these neural tissues per chimeric embryo varied from 3065 to 11295 (Table 1), and showed no correlation with any particular combination of graft and recipient.

Correlation of distribution pattern of quail cells in chimeras with the neural axis level of donor embryos at which grafts were obtained

In the DRG of 4.5-day chimeras, the ratio of quail cells derived from the neural crest at 15-20 somite levels from quail embryos at 24 to 29-somite stages to total quail cell number in the chimera gradually decreased from about 50% to 20% in a rostral to caudal order. Such ratio was almost constant at about 20% when transplanted cells were obtained from the 21-30 somite levels of 24 to 38-somite stage embryos, and gradually increased from about 20% to 50% in a rostral to caudal order with grafts obtained from the 31-35 somite levels of 39 to 42-somite stage quail embryos (Fig. 4A). Conversely, in sympathetic tissues of 4.5-day chimeras, the ratio of quail cells derived from the neural crest at 15-20 somite levels to total quail cell number gradually increased from about 30% to 70% in a rostral to caudal order. This ratio was virtually constant at about 70% with the grafts from the 21-30 somite levels, while it decreased gradually from 70% to 30% in a rostral to caudal order with the grafts from the 31-35 somite levels (Fig. 4B). The ratio of quail cells within the spinal nerve to total quail cell number remained virtually constant at about 20% throughout all rostrocaudal levels examined (Fig. 5A).

Thus, the pieces of neural crest obtained from a certain rostro-caudal level of 2.5-day quail embryos gave rise to cells in three kinds of neural tissues, DRG, ST and the spinal nerve, at a certain ratio in 4.5-day chimeras. That is, the distribution pattern of transplanted quail neural crest cells differed according to the level in the quail embryo from which the grafts were originally obtained.

Correlation of distribution patterns of quail cells in chimeras with rostrocaudal axis level of grafted site in host embryo

To determine the effect of the microenvironment on the development of the quail neural crest transplanted into chick embryos, we classified the chimeras into three groups according to the somite level of the host where the quail transplants were insert-

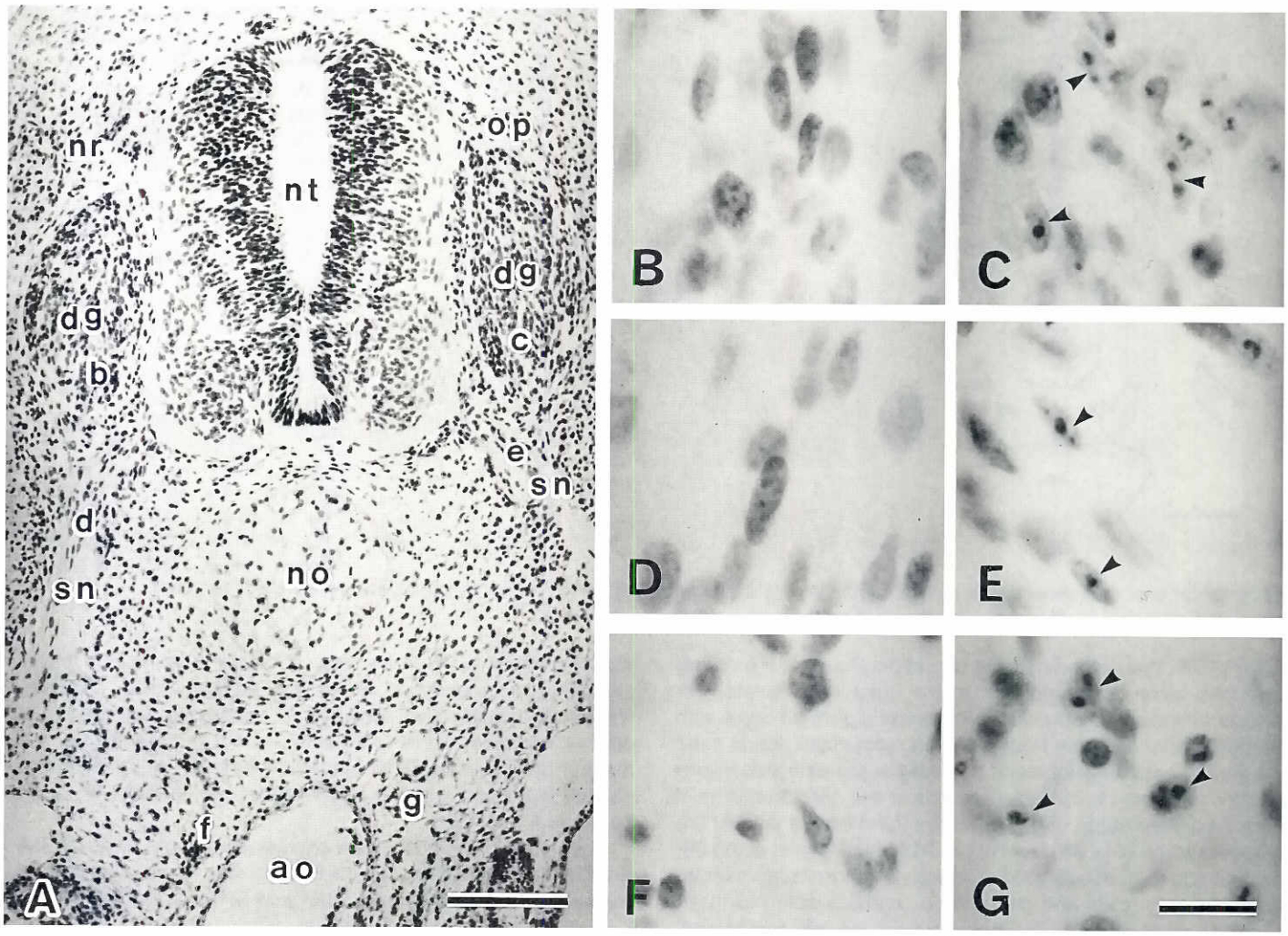

Fig. 2. Photomicrographs of chimeric embryo. (A) *Transverse section of 4.5-day-old chimeric embryo, 2 days after the operation stained by Feulgen's method. The regions enlarged in* **(B-G)** *are indicated by the corresponding lower-case characters. nt: neural tube, no: notochord, sn: spinal nerve, ao: dorsal aorta, dg: dorsal root ganglion (DRG), nr: normal side, op: operated side.* **(B,D,F)** *Higher magnification of the normal (left) side DRG (b), spinal nerve (d) and sympathetic tissue (around the aorta) (f), respectively.* **(C,E,G)** *Higher magnification of the operated (right) side DRG(c), spinal nerve (e) and sympathetic tissue (g), respectively. Each tissue derived from the neural crest on the operated (chimeric) side* **(C,E and G)** *contained many quail cells with massed heterochromatin in their nuclei (arrowheads), whereas no quail cells were seen in any tissue on the normal side* **(B,D and F)**. *Bar: A, 100 μm; B-G, 25 μm.*

ed. These three levels were rostral to, equal to, and caudal to, the adrenomedullary level (18-24 somite level) of chick embryos.

In all three groups, the distribution patterns of quail cells were essentially the same as those outlined above. That is, quail neural crest cells obtained from brachial (somite level 15-17) or lumbosacral somite levels (somite level 25-34) were distributed mainly in the DRG, and those from the adrenomedullary level (somite level 18-24) were distributed less in the DRG (Fig. 6). In contrast, quail neural crest cells derived from adrenomedullary levels were distributed mainly in sympathetic tissues, while the distribution of those cells from brachial or lumbosacral levels in these tissues was less (data not shown).

The host somite level where the quail neural crest fragment was transplanted was not correlated with the distribution pattern of quail cells among the nervous tissues, which were in the DRG (Fig. 7A), the sympathetic tissues (Fig. 7B) and spinal nerves (Fig. 5B).

Thus, the distribution pattern of transplanted quail neural crest in chimeric embryos was correlated with the somite level from which the neural crest was obtained but not with the region into which the graft was transplanted, suggesting that the fate of neural crest had been restricted in the ratio of distribution in the formation of DRG, ST and spinal nerves according to its neuraxis level.

Developmental stage of the quail neural crest at transplantation

It is possible that our findings might have been due to differences in developmental stages of the neural crest used as grafts, since the fate of neural crest cells has been shown to be determined by the timing of onset of their migration (Serbedzija

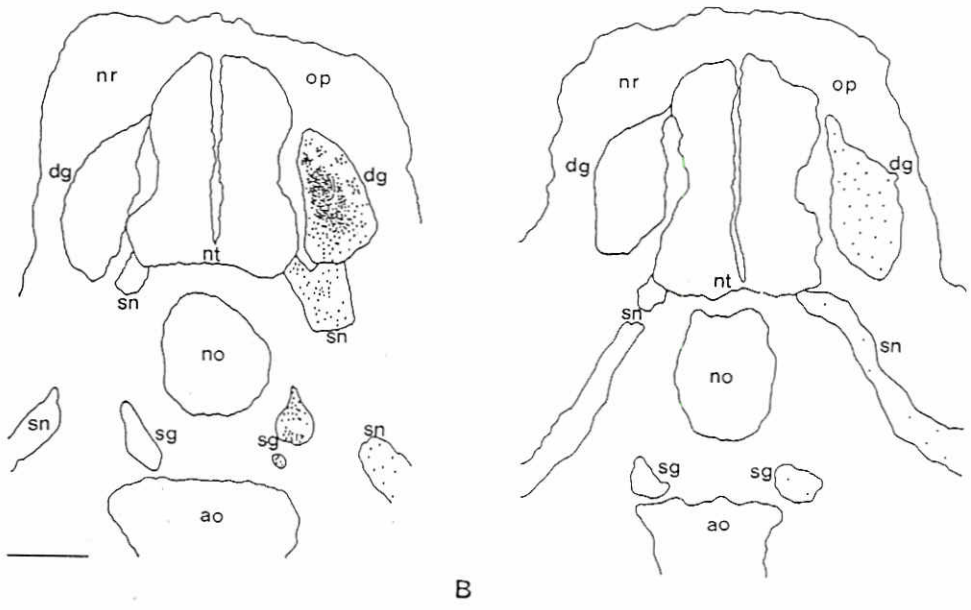

Fig. 3. Tracing of transverse sections of two 4.5-day-old chimeric embryos. • *indicates a quail cell. nt: neural tube, no: notochord, sn: spinal nerve, ao: dorsal aorta, dg: DRG, sg: sympathetic ganglion, nr: normal side, op: operated side.* **(A)** *Chimeric embryo containing a relatively large number of quail cells (total number of quail cells, 9576: specimen No 6 in Table 1).* **(B)** *Chimeric embryo containing a relatively small number of quail cells (total number of quail cells, 3887: specimen No 9 in Table 1). In each chimera, transplants were obtained from the brachial region of quail embryos. In both A and B, the chimeric DRG, spinal nerve and sympathetic ganglion on the operated (right) side contained quail cells. No quail cells were seen in any tissue on the normal (left) side. Although the total numbers of quail cells varied (range, 3065 to 11295), the distribution pattern of quail cells derived from a given somite level in the three tissues did not differ from embryo to embryo. Bar, 100 μm.*

et al., 1989). We determined the migration phase of the neural crest cells which corresponded to the crest cells isolated for transplantation as described above in normal quail embryos with the monoclonal antibody HNK-1, which recognizes neural crest cells during early development, to visualize the extent of migration. We examined transverse sections at the 16th somite level of the 24-somite stage (Fig. 8A), at the 22nd somite level of the 30-somite stage (Fig. 8B), and at the 28th somite level of the 36-somite stage (Fig. 8C) of quail embryos, which were representative of the crest cells and the embryos corresponding to those used for transplantation. The quail neural crest cells had migrated more or less at the same extent between the neural tube and the somites, and had not entered the sclerotome in any sections examined, indicating that the neural crest cells used as grafts were at a similar phase of migration.

Size variation of DRG along the rostrocaudal axis in a normal embryo

The difference of distribution ratio of transplanted crest cells along rostrocaudal axis of donor quail embryo may reflect the size of each neural rudiment. Although the neural crest at the 18-24th somite level has been shown to give rise to the adrenal medulla (Le Douarin and Teillet, 1974; Le Douarin, 1982), the size of DRG rudiments at each rostrocaudal level has not been measured. We counted the number of cells forming the DRG at various somite levels in normal developing quail embryos at 4.5 days (n= 6) and 5 days (n= 4) by determining the cell number in 7 μm serial sections.

In the 4.5-day-old quail embryos, the number of cells in the DRG at the brachial level was significantly higher than those at more rostral and more caudal levels ($P<0.01$; t-test, n=6, Fig. 9A). At levels other than the brachial level, however, the numbers of cells in the DRG were almost equal.

In this study, grafts from the lumbosacral level were obtained from embryos that were about half a day older than those from which brachial level grafts were obtained. That is, the transplanted quail lumbosacral neural crest cells in 4.5-day chimeras were considered to be 5 days old. Consequently, we investigated cell numbers in the DRG of 5-day-old quail embryos; the number of cells in the DRG at the lumbosacral and brachial levels was significantly higher than at other somite levels ($P<0.01$; t-test, n= 4, Fig. 9B).

Thus, the DRG rudiments consisted of more cells at the brachial and lumbosacral levels than at other levels, even before natural cell death had begun (Carr and Simpson, 1978).

Discussion

Transplantation of isolated neural crest

In this study, we showed that the distribution pattern of the derivatives of transplanted quail trunk neural crest in quail-chick chimeras in three kinds of tissues differed according to the rostrocaudal axis level from which the grafts were obtained, and these differences were not affected by their circumstances in host chick embryos.

It may be presumed that difference in cell-cell adhesion between chicken and quail cells may affect our results and it may be advisable to use homochronic, heterotopic chicken-chicken transplantation experiments with labeled chicken neural crest cells. We consider, however that this seems contradictory. The different characteristics of cell adhesion between chick and quail may not cause important effects if cell adhesion is uniform overall. On the contrary, if the difference of adhesion changed, for example, along rostrocaudal axis, it is possible that the adhesiveness may be different along the rostrocaudal axis in an embryo. In such a case, we should consider the effect of differential adhesiveness on the heterotopic transplantation even in a chimera.

We consider our results that the graft obtained from a certain rostrocaudal level contributes to the formation of rudiments of

TABLE 1

SUMMARY OF OPERATIONS AND RESULTS

SP NO	LEV/ST OF DN	LEV/ST OF HT	TQ	DQ	FQ	SQ	DQ/TQ	FQ/TQ	SQ/TQ
1	15/28	30/30	5160	2706	1188	1266	52.36	23.02	24.53
2	16/24	10/18	3895	1195	652	2084	30.68	16.74	52.58
3	16/24	12/21	6184	2213	1545	2462	35.79	24.98	39.23
4	16/24	13/17	4219	1997	649	1573	47.33	15.38	37.28
5	16/24	16/20	6531	3681	930	1920	56.36	14.24	19.40
6	16/24	20/21	9576	5592	1543	2441	58.40	16.11	25.49
7	16/24	25/25	4020	1248	474	2298	31.04	11.79	57.16
8	16/24	25/26	5444	2300	1708	1436	42.25	31.37	26.38
9	16/24	27/27	3887	1810	807	1270	46.57	20.76	32.67
10	17/25	25/26	4745	2544	561	1640	53.61	11.82	34.57
11	18/26	21/22	3425	1182	1191	1052	34.51	34.77	30.72
12	18/26	27/28	4495	1171	753	2571	26.05	16.75	57.20
13	18/26	28/29	5216	612	403	4201	11.73	7.73	80.54
14	18/26	29/32	4956	1823	1213	1920	36.78	24.48	38.74
15	18/26	33/33	3924	1195	1224	1505	30.45	31.19	38.35
16	19/27	18/29	3572	1144	1030	1398	32.03	28.84	39.14
17	19/27	23/25	5982	1783	1153	3046	29.81	19.27	50.92
18	19/27	25/25	4753	1562	654	2537	34.16	13.76	53.38
19	19/27	25/26	11295	2680	3054	5561	23.73	27.04	49.23
20	19/27	27/27	5462	1095	1562	2805	20.05	28.60	51.35
21	19/27	27/28	5923	1854	1694	2375	31.30	28.60	40.10
22	19/28	17/18	5305	1988	1041	2276	37.47	19.62	42.91
23	19/28	28/28	4886	1558	1098	2230	31.89	22.47	45.64
24	20/28	20/22	3883	134	679	3070	3.45	17.49	79.06
25	20/28	20/22	3750	802	414	2534	21.39	11.04	67.57
26	20/29	19/20	7015	1702	1162	4241	24.26	16.56	60.46
27	21/24	24/26	3258	518	511	2229	15.90	15.69	68.41
28	21/24	26/27	3065	383	300	2385	12.50	9.79	77.81
29	21/29	19/20	3318	662	321	2325	19.95	9.67	70.07
30	21/29	19/24	5503	1165	1365	2974	21.17	24.80	54.03
31	21/29	30/32	5943	1508	986	3449	25.37	16.59	58.03
32	22/30	18/25	3682	549	557	2576	14.91	15.13	69.96
33	22/30	18/25	8561	2065	1874	4622	24.12	21.89	53.99
34	22/30	20/22	9337	2688	1544	5105	28.79	16.54	54.67
35	22/30	21/24	10182	2354	2506	5326	23.12	24.57	52.31
36	22/30	25/26	8262	1968	1987	4307	23.82	24.05	52.13
37	23/31	23/24	4058	965	930	2163	23.78	22.92	53.30
38	23/31	24/25	4540	1115	371	3054	24.56	8.17	67.27
39	23/31	25/25	3990	512	1167	2311	12.83	29.25	57.92
40	24/27	22/23	3257	381	697	2182	11.70	21.40	66.99
41	24/32	16/17	4147	622	836	2689	15.00	20.16	64.84
42	24/32	20/23	4688	1019	204	3465	21.74	4.35	73.91
43	24/32	23/23	3946	1218	625	2103	30.87	15.84	53.29
44	25/33	22/24	4898	741	1053	3104	15.13	21.50	63.37
45	25/33	23/25	3765	849	894	2042	22.43	23.62	53.95
46	25/33	23/25	7451	739	1013	5699	9.92	13.60	76.49
47	25/33	30/31	8221	1065	712	6444	12.95	8.66	78.38
48	26/34	17/18	3411	305	445	2661	8.94	13.05	78.01
49	26/34	21/22	5441	351	2271	2891	6.45	41.74	51.81
50	27/35	24/25	5279	805	725	3749	15.25	13.73	71.02
51	27/35	24/26	11125	2823	2304	5998	25.38	20.71	53.91
52	27/35	25/26	4220	596	1063	2561	14.12	25.19	60.69
53	28/36	22/22	3356	281	751	2324	8.37	22.38	69.25
54	28/36	25/26	10747	2426	2298	6023	22.57	21.38	56.04
55	28/36	32/33	4713	316	596	3801	6.70	12.65	80.65
56	30/38	23/24	8639	2965	2240	3434	34.32	25.93	39.75
57	30/38	23/25	3827	1042	1581	1204	27.23	41.31	31.46
58	30/38	25/26	6176	1223	1956	1997	19.80	31.67	48.53
59	31/39	17/18	5536	2065	1846	1625	37.30	33.35	29.35
60	31/39	25/26	5779	1295	2284	2200	22.41	39.52	38.07
61	32/40	24/25	6894	3136	1495	2263	45.49	21.69	32.83
62	32/40	24/25	6253	2518	1956	1779	40.27	31.28	28.45
63	32/40	24/25	9782	3516	2964	3302	35.94	30.30	33.76
64	33/41	16/17	10255	5256	2092	2907	51.25	20.40	28.35
65	33/41	26/27	9093	4963	2264	1866	54.58	24.90	20.52
66	34/42	23/24	7242	3849	1192	2201	53.15	16.46	30.39

Abbreviations: sp No, specimen number; lev/st of dn, donor somite level from which transplant was obtained and donor stage indicated by number of somites; lev/st of ht, host somite level into which transplant was inserted and host stage indicated by number of somites; TQ; total quail cell number in a chimeric embryo; DG, quail cell number in DRG; FQ, quail cell number in spinal nerve; SQ, quail cell number in sympathetic tissue; DQ/TQ, ratio of quail cell number in DRG to total quail cell number in a chimera; FQ/TQ, ratio of quail cell number in spinal nerve to total quail cell number in a chimera; SQ/TQ, ratio of quail cell number in sympathetic tissue to total quail cell number in a chimera.

each nervous tissue at the same distribution ratio, having no relation to the level of transplantation in host, showed the essential potency of grafted neural crest, overcoming the problem such as differential adhesiveness between chicken and quai and/or between transplantation sites.

In fact, chick cells and quail cells behave differently in chimeric embryos. More cells from quail somites transplanted in chick embryos participated in limb muscle formation than from chick transplants in quail embryos (Chevallier *et al.*, 1977). This may be the result of faster migration and/or proliferation of quail cells than chick cells. However, this fact suggests that quail crest cells might express more their own potency of migration when they are transplanted in chick embryos, than chick crest cells when they are transplanted in quail embryos. This allows us to evaluate the potency of crest cell migration and/or proliferation.

The result in this study was obtained by transplantation of the isolated neural crest from quail embryos into chick embryos instead of using neural crest with the neural tube as transplants. Transplantation of the isolated neural crest can avoid the influence of the donor neural tube on gangliogenesis, which was, for example, shown by Kalcheim and Le Douarin (1986), and may allow the neural crest cells to express their own potencies under the influence only of host tissues including host neural crest cells.

Moreover, this experimental system allowed us to quantitatively examine the developmental potential of neural crest at a given somite level by describing the distribution patterns of the descendants of transplanted quail neural crest cells as the ratio of quail cells found in each chimeric tissue to the total quail cells in the chimera. It should be noted that we quantified the potential of crest cells to form each tissue and not that of differentiation. That is, our assay represented a combination of the selection of migration pathways and cell growth at the settled sites. Since even some crest cells, for example, those forming the dorsal root ganglia, have the potential to differentiate into adrenergic neurons and pigment cells (Sieber-Blum *et al.*, 1993), cell differentiation and selection of migration pathway must be controlled differently and independently. Such independence of cell differentiation and morphogenesis was also shown in the skeletogenesis from somites (Aoyama and Asamoto, 1988; Aoyama, 1993). The determination of the morphology of the axial skeleton was shown to precede that of somite cell differentiation into sclerotome or dermomyotome.

Restriction of the developmental fate of the neural crest along rostro-caudal axis

Le Douarin and Teillet (1973), by transplanting the vagal neural crest into the truncal level and vice versa, showed that heterotopically implanted neural primordium produced crest cells which behaved in virtually the same way as indigenous cells. On the other hand, when the cephalic neural crest was implanted into the cervicotruncal level, it formed cartilaginous and connective tissue in the dermis and vertebrae (Le Douarin and Teillet, 1974) which is not formed from the cervicotruncal crest but from somites (Le Lièvre and Le Douarin, 1975). Thus, although the neural crest is pluripotent, the variety of its derivatives is restricted to a certain range according to the neuraxis level. Our present findings suggest that restriction of the developmental fate in gangliogenesis varies within trunk regions along the neuraxis. This might be inter-

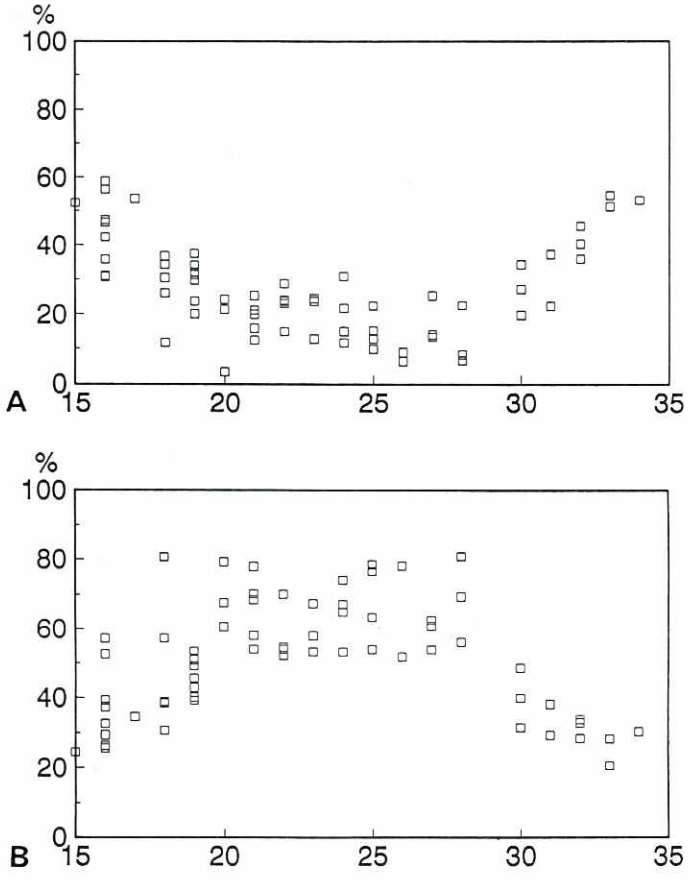

Fig. 4. Relationship between somite level in donor from which transplants were obtained and ratios of the number of quail cells in DRG (A), or sympathetic tissue (B) to total quail cell number in a chimeric embryo. *Numbers on the abscissa indicate somite level in the donor, indicated by somite numbers. The settlement pattern of the derivative cells from transplanted trunk neural crest in the early migration phase differed along the rostrocaudal axis.*

preted as indicating that the ratio of three kinds of stem cells which migrate and settle to participate in the formation of DRG, ST and spinal nerve, respectively, is different along the truncal neuraxis. As shown by clonal culture (Bronner-Fraser *et al.*, 1980; Sieber-Blum and Cohen, 1980; Baroffio *et al.*, 1988; Sieber-Blum, 1989; Dupin *et al.*, 1990; Deville *et al.*, 1992) and by single cell labeling (Bronner-Fraser and Fraser, 1988, 1989), neural crest is composed of subpopulations with different repertoires of cell differentiation potentials. Alternatively, common precursor cells of DRG, ST and nerve cells might be determined to produce these cells at a specific ratio according to the neuraxis level.

The ratio of cells distributed in the 'sympathetic tissue'

It seems inconsistent that 70% of transplanted quail cells derived from the 21-30 somites level were distributed in the sympathetic tissue because the DRG is generally larger than the sympathetic ganglia. However, we could not distinguish the adrenomedullary cells from cells in the sympathetic trunk, because the latter were not well aggregated at 4.5-days of incubation and were scattered together with the adrenomedullary cells around the dorsal aorta. This means that 70% of quail cells were included with the cells forming not only the sympathetic trunk but also the adrenal medulla. That the neural crest cells derived from the 21-30 somites level tended to form sympathetic tissues may reflect their ability to form adrenal medulla which is formed by neural crest cells at the 18-24 somites level.

Environmental regulation in gangliogenesis

We have previously shown that the size of the DRG is regulated by the environment. That is, using the same experimental system as in the present study, we showed that the transplantation of additional quail neural crest cells did not affect the size of DRG (Asamoto *et al.*, 1992), which is apparently inconsistent with the present results. This raises the question as to what made the quail cells develop according to their own fate under the influence of the host environment; this might be explained by higher growth rate of quail cells than of chick cells, or by the site of transplantation which the host neural crest had not reached yet. In either case, the quail neural crest cells preceded the host chick cells in the formation of ganglia. It is likely that the surrounding tissue regulates the size of ganglia at a later stage of

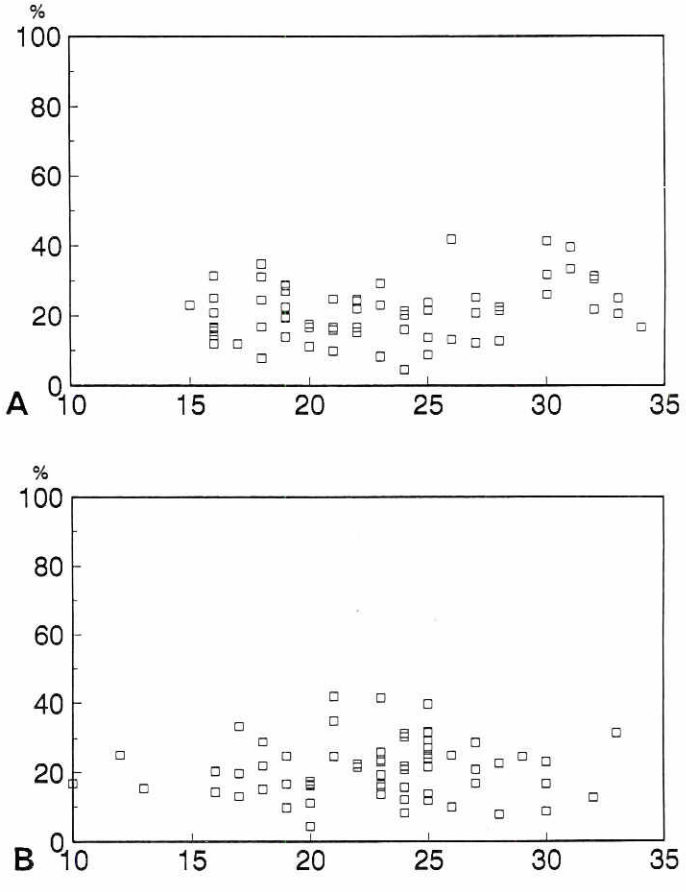

Fig. 5. Correlation between donor somite level from which transplants were obtained (A) or host somite level at which isolated crest cells were transplanted (B) and the ratio of quail neural crest cells within the spinal nerve to total cell number in a chimeric embryo. *Quail cells were distributed almost at a constant ratio within the spinal nerves, regardless of the donor or host somite levels.*

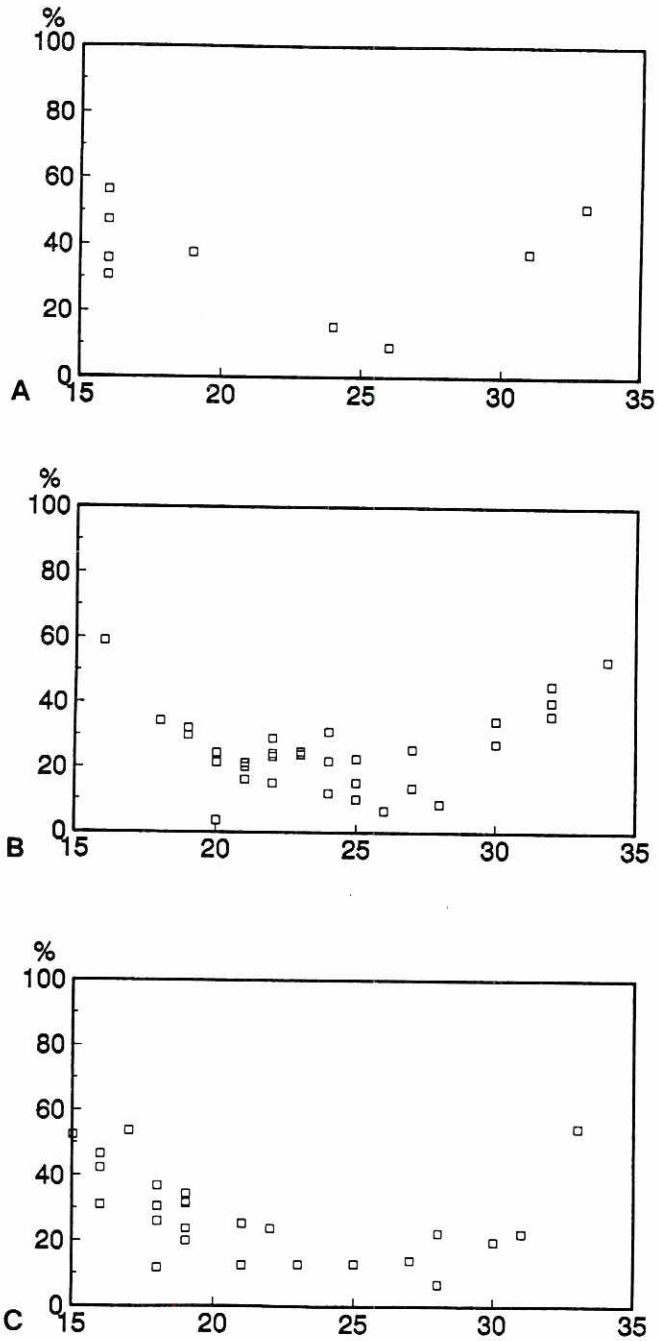

Fig. 6. Correlation between the settlement sites of transplanted crest cells and the site of transplantation. *Numbers on the abscissa indicate donor somite levels from which transplants were obtained. The ordinate shows the ratio of quail cell numbers in the DRG to total quail cell numbers in a chimeric embryo. Chimeric embryos were divided into 3 groups according to host (chick) somite levels at which quail neural crest cells were transplanted.* **(A)** *Chimeras in which quail neural crest cells were transplanted into somite levels rostral to adrenomedullary levels.* **(B)** *Those in which transplants were made into adrenomedullary (18-24 somite) levels.* **(C)** *Those in which transplants were made into somite levels caudal to adrenomedullary levels. There is virtually no difference among the profiles of graphs A, B and C. Thus, the quantitative differences of settlement sites of transplanted crest cells along the original rostrocaudal axis were not affected by the host environment.*

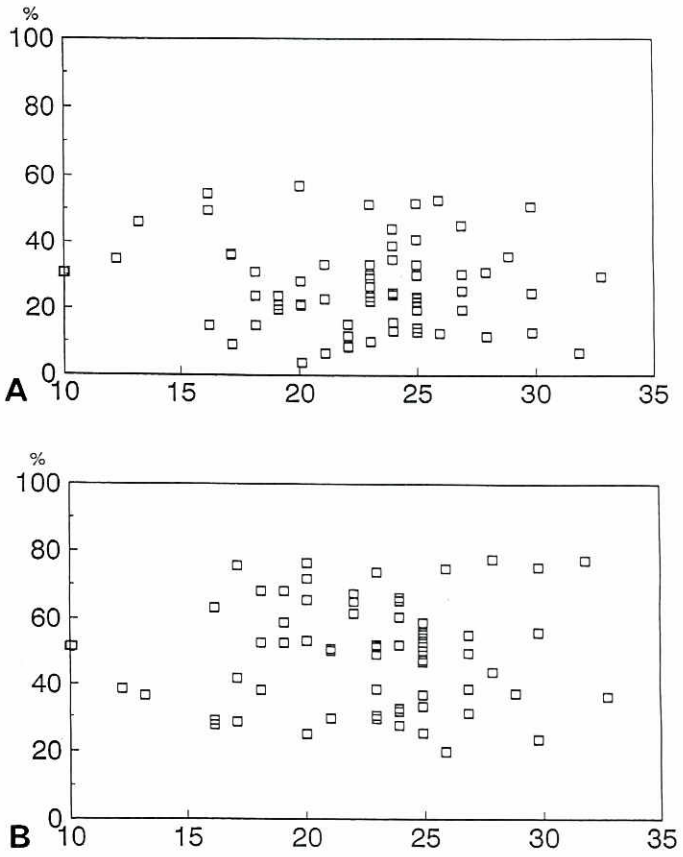

Fig. 7. Correlation between the transplantation site in host embryos and the ratio of quail neural crest cells in the DRG (A) and ST (B) to total quail cell numbers in a chimeric embryo. *Numbers on the abscissa indicate host somite levels to which quail neural crest cells were transplanted. There was no correlation between these two factors in A or B.*

gangliogenesis. If this is the case, host chick cells should be affected more strongly than quail cells, and the number of chick cells might thus be preferentially reduced.

We still have to explain the discrepancy between the indigenous fate of neural crest, which was shown to be unchangeable by the host circumstances, and the regulative formation of ganglia. Probably, the size of the ganglion was doubly guaranteed by the potency of neural crest as a source and by the somites, for example, as an environmental regulator of gangliogenesis. Although occipital neural crest formed spinal ganglia which are not formed normally after transplantation of occipital neural tube into the truncal region, these spinal ganglia were smaller than normal trunk spinal ganglia (Lim *et al.*, 1987). In this case also both neural crest cells themselves and their environment restrict ganglion size.

We showed that the rudiments of the DRG were larger at the brachial and lumbosacral levels than at other levels, even before the onset of natural cell death. Oppenheim *et al.* (1989) found significantly more motoneurons in the brachial and lumbar segments than in other segments on embryonic day 4, which is before the onset of cell death and secondary migration of neurons out of the motor column. Thus, in the case of both DRG and

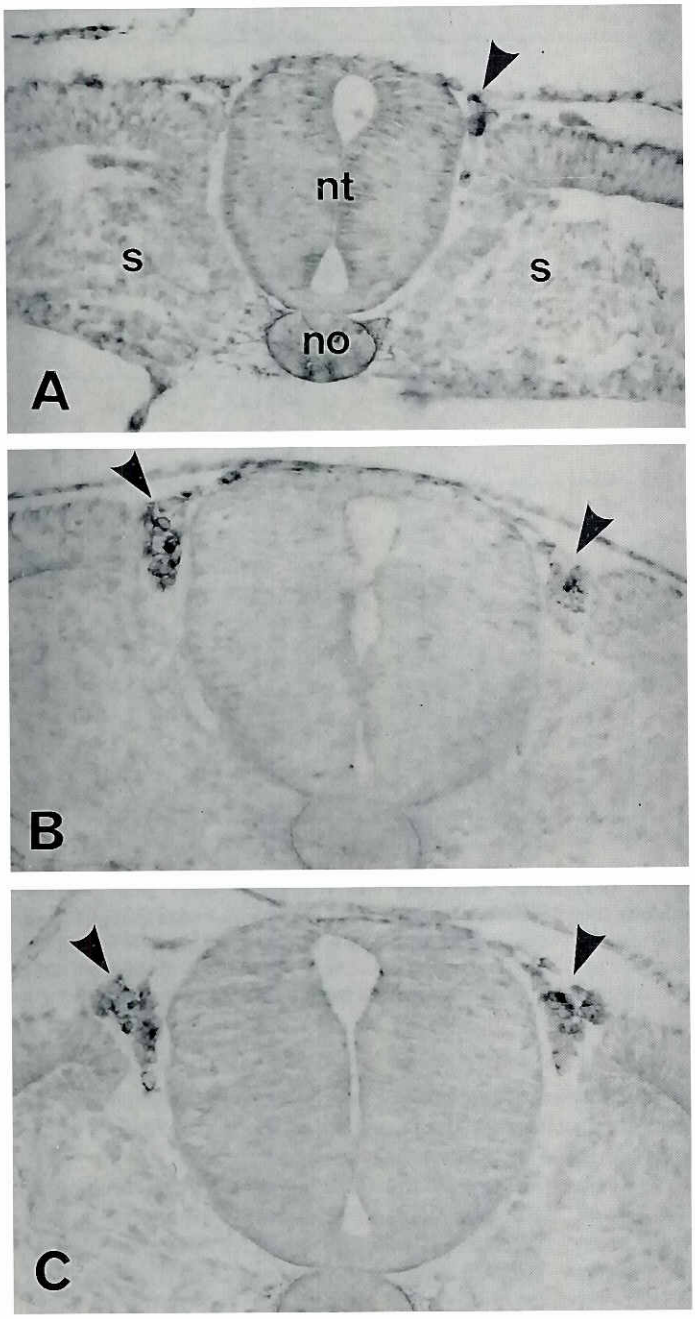

Fig. 8. Migration phase of quail neural crest cells for transplantation at various somite levels, shown in transverse sections stained with monoclonal antibody HNK-1. *nt: neural tube, no: notochord, s: sclerotome.* **(A)** *16 somite level of 24-somite stage embryo.* **(B)** *22 somite level of 30-somite stage embryo.* **(C)** *28 somite level of 36-somite stage embryo. At these somite levels, quail neural crest cells (arrowheads) migrated between the neural tube and somite, and did not enter the sclerotome. Bar, 50 μm.*

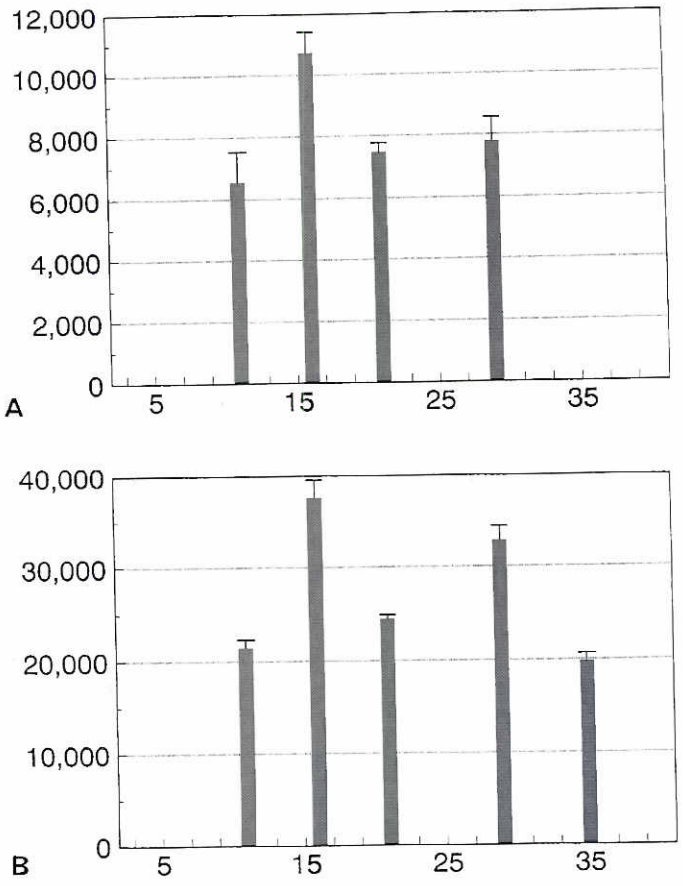

Fig. 9. Mean total cell number and S.D. in the DRG at various somite levels in 4.5-day (A) and 5-day (B) quail embryos. *Numbers on the abscissa indicate somite level, shown by somite numbers. The DRG at the brachial level (bar on somite number 16 in* **(A),** *in the 4.5-day embryo, and the DRG at the brachial (bar on somite number 16 in* **(B),** *and lumbosacral levels (bar on somite number 29 in* **(B),** *in the 5-day embryo included significantly more cells than the DRG at other somite levels (P<0.01, t-test). Sample size: 6 for each data point in the 4.5-day embryo and 4 for each point in the 5-day embryo.*

motor column, there are initial differences in size of precursor cell populations along the rostrocaudal axis, although cell death may contribute to the later regional variation in cell numbers. The finding that the brachial and lumbar neural crest of quail embryos tended to produce more DRG cells than ST cells in chimeras may reflect the difference in size of DRG rudiments. However, the peak of the ratio of cells participating in the formation of DRG was shifted more caudally than the lumbar level. This may be due to the loss of neural crest cells forming adrenal medulla at the 31-35 somite levels and the relative ratio of cell numbers of quail cells producing DRG cells to total quail cell numbers was increased in these regions. We cannot say which contributed more to the apparent potential difference of crest cells along the rostrocaudal axis, potency of DRG cells or adrenomedullary cells, since we did not find any consistent difference in the absolute value along the neuraxis but in the relative ratio of forming DRG, ST and spinal nerve.

Materials and Methods

Embryos

Fertilized White Leghorn and Japanese quail eggs were purchased from a local farm. The eggs were incubated at 38°C for 2.5 to 3 days,

reaching the 17-33 somite stage in chick embryos and the 24-42 somite stage in quail embryos.

Isolation of quail neural crest

After sterilizing the surface of the quail egg shell with 70% ethanol, we broke the egg into Ca^{2+}- and Mg^{2+}-free phosphate buffered saline (CMF) in a Petri dish, where the embryo was removed from the yolk. The part of the embryo containing the neural tube, corresponding to the most caudal 7th to 10th somites, was cut out in CMF, using a scalpel under a dissecting microscope. The fragment was incubated in 500 i.u./ml Dispase (Godo Shusei Co. Ltd, Tokyo, Japan) in Dulbecco's modified Eagle's MEM (Nissui, Tokyo, Japan) supplemented with 10% fetal calf serum (Bocknek, Rexdale, Ontario, Canada) (DMEM) at 37°C for 20-30 min, and then the neural tube was isolated from the surrounding tissues with tungsten needles sharpened with molten sodium nitrite. The neural crest on the dorsolateral surface of the neural tube was peeled off as small fragments with a pair of sharpened tungsten needles. These fragments of the quail neural crest were transferred into a drop of DMEM with a micropipette and kept at room temperature until they were transplanted into chick embryos.

Transplantation

Transplantation of quail neural crest fragments into chick embryos was carried out *in ovo*, through a window in the egg shell. A slit was made between the neural tube and the most caudal 3 somites with a microscalpel made from a sewing needle. Into this slit we inserted the fragment of quail neural crest with the microscalpel. After this operation, the window of the host egg shell was sealed with cellophane tape and the egg was further incubated at 38°C (Fig. 1).

Histology

Quail-chick chimeric embryos were fixed with 3.5% formalin in 0.1 M phosphate buffer (pH 7.4) 2 days after the operation at stage 25 (Hamburger and Hamilton, 1951). Since natural cell death in the DRG begins after this stage, we need not consider the effect of the decrease in number of cells in the DRG caused by cell death (Carr and Simpson, 1978). The embryos were dehydrated, embedded in paraffin and 7 μm-thick serial transverse sections were prepared. The descendants of the transplanted quail cells can be distinguished from the host chick cells after the Feulgen and Rossenbeck reaction (Le Douarin, 1973). Quail heterochromatin was massed in the nucleus, while chick heterochromatin was more uniformly dispersed (Fig. 2). The nuclei of the quail cells in the chimeric DRG, spinal nerve and around the dorsal aorta (sympathetic tissue) were traced with the aid of a camera lucida (Fig. 3). The numbers of quail cells in each tissue were summed for one embryo, and the ratio of quail cells in each tissue to the total number of quail cells in one embryo was calculated. The numbers of cells determined in this way did not represent actual cell numbers but were sometimes as large as actual numbers, because some nuclei were cut and counted in two adjacent sections. However, this occurred equally in cells of all three tissues. The ratio of numbers of quail cells in three tissues to total numbers of quail cells calculated as above is equal to that obtained from the actual number of quail cells. It should be noted that the numbering of somite levels from which quail transplants were obtained was slightly complicated. We obtained transplants from the dorsal surface of neural tube of 4-somite length. As the transplants were obtained as some small fragments from one neural tube, we could not know where the small fragment of neural crest was situated on 4-somite length neural tube. We use, therefore, the second somite number of the 4-somites length as the somite level from which quail transplants were obtained.

To determine the migration phase of the quail neural crest used for transplantation, we performed immunohistochemical studies of transverse sections of 2- to 3-day quail embryos using the monoclonal antibody HNK-1 (Tucker *et al.*, 1984; Funakoshi Co. Ltd, Tokyo, Japan; 1:10 dilution with phosphate-buffered saline containing 0.3% bovine serum albumin). Sections were prepared by the same procedure as that used for the chimeras, except that we used Carnoy fixative. After incubation with HNK-1 for 1 h at room temperature, the sections were processed using a Histofine SAB-PO kit (Seikagaku Co. Ltd, Tokyo, Japan), and visualized with 3,3'-diaminobenzidine.

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