INTRODUCTION

During avian gastrulation, strips of apparently unsegmented paraxial mesoderm, called the segmental plates, are formed on either side of the notochord. The cells that constitute these segmental plates are derived from the presumptive somite region of the epiblast (Rosenquist, 1966; Vakaet, 1984). They join the caudal ends of the segmental plates after involuting through the cranial end of the primitive streak (Rosenquist, 1966; Vakaet, 1984; Nicolet, 1970, 1971; Packard, 1986a). While somites segment in successive pairs from the cranial ends of the segmental plates, the morphogenetic movements associated with gastrulation continue to add new cells to the caudal ends of the segmental plates at least until the 8-somite stage of development (Packard, 1986a). These morphogenetic movements and some cell divisions within the segmental plate approximately compensate for the cells lost in segmentation, although the segmental plates do vary somewhat in length during much of development (Packard and Jacobson, 1976; Packard, 1978; Sandor and Fazakas-Todea, 1980) indicate that the spatiotemporal pattern of somite formation reflects a stable covert organization (i.e., a prepattern) inherent to the segmental plates and extending along their entire length. Furthermore, morphological studies (Meier, 1979, 1982a,b; Triplett and Meier, 1982; Packard and Meier, 1983) have shown that this prepattern can be distinguished as a series of paired circular domains, termed somitomeres, within the segmental plates. The somitomere pattern represents the segmental pattern that the segmental plate cells are specified to form (Jacobson, 1992). The results of these aforementioned studies clearly demonstrate that, for most of the period of somitogenesis, the segmental plates contain at least some cells that together are developmentally specified to form a defined pattern of about 10 somites in a cranial-to-caudal sequence. These studies also strongly suggest that the commitment of cells to form elements of the pattern is acquired prior to the cells entering the caudal end of the segmental plate. Indeed, reversal of the cranial-caudal orientation of prospective paraxial mesoderm lying caudal to the node, that is, before the cells have entered the segmental plate, leads, hours later, to a caudal-to-cranial sequence of segmentation (Christ et al., 1974; also see review by Davidson, 1988). Furthermore, somito-

SUMMARY

Previous experimental evidence suggested that the avian segmental pattern is already specified in the apparently unsegmented paraxial (segmental plate) mesoderm, but is susceptible to modification and reconstitution. We explored capacities of embryos to alter the specified pattern and restore it after disruption. In control experiments, right segmental plates of chicken or Japanese quail embryos were removed after about 48 hours of incubation and immediately replaced. Hensen's node and the primitive streak were removed to halt further segmental plate formation and the embryos were cultured for about 18 hours more. Somite numbers on the operated and unoperated sides were nearly identical ($r=0.904$, $n=31$, $P<0.001$); no species differences were noted. Right segmental plates of chicken hosts were then replaced with right segmental plates from quail donors. The numbers of somites formed by donors and grafts were not significantly correlated ($r=0.305$, $n=30$, $P<0.1$), but the correlation between the graft and the host’s unoperated side was significant ($r=0.666$, $n=30$, $P<0.001$). The host is therefore able to alter the number of somites formed by the graft to one more compatible with the host’s pattern. From orthostereoscopic reconstructions, it appeared that the location and size of somites could also be adjusted by the host. Similar results were obtained for tandem grafts of anterior halves of segmental plates and for grafts of minced segmental plates, though in the latter case contact with tissues near the midline was necessary for somite formation.

Key words: segmentation, segmental plate, mesoderm, somite, pattern formation, chick, quail

INTRODUCTION

Somite pattern regulation in the avian segmental plate mesoderm

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Although the segmental plates of living embryos appear to be unsegmented, a series of experimental studies (Menkes and Sandor, 1969; Christ et al., 1974; Packard and Jacobson, 1976; Packard, 1978; Sandor and Fazakas-Todea, 1980) indicate that the spatiotemporal pattern of somite formation reflects a stable covert organization (i.e., a prepattern) inherent to the segmental plates and extending along their entire length. Furthermore, morphological studies (Meier, 1979, 1982a,b; Triplett and Meier, 1982; Packard and Meier, 1983) have shown that this prepattern can be distinguished as a series of paired circular domains, termed somitomeres, within the segmental plates. The somitomere pattern represents the segmental pattern that the segmental plate cells are specified to form (Jacobson, 1992). The results of these aforementioned studies clearly demonstrate that, for most of the period of somitogenesis, the segmental plates contain at least some cells that together are developmentally specified to form a defined pattern of about 10 somites in a cranial-to-caudal sequence. These studies also strongly suggest that the commitment of cells to form elements of the pattern is acquired prior to the cells entering the caudal end of the segmental plate. Indeed, reversal of the cranial-caudal orientation of prospective paraxial mesoderm lying caudal to the node, that is, before the cells have entered the segmental plate, leads, hours later, to a caudal-to-cranial sequence of segmentation (Christ et al., 1974; also see review by Davidson, 1988). Furthermore, somito-
moters have been observed caudal to the node in the newly formed mesoderm that has not yet entered the segmental plates (Meier and Jacobson, 1982; Packard and Meier, 1983; Jacobson and Meier, 1986).

The experiments reported in this paper were inspired in part by findings that, in certain cases, suggest that the somitic prepattern within the segmental plate is susceptible to permanent modification yet, in other cases, suggest that the original specified pattern may be restored after thorough disruption. Heat-shock treatment of chick embryos on the second day of incubation causes reproducible somite pattern anomalies (Pirmett et al., 1988; 1989; Veini and Bel-lairs, 1986). Many of these anomalies occur 6 to 7 somites caudal to the last somite pair to form prior to the experiment. Since the chick segmental plate contains 10 to 12 prospective somites (Packard and Jacobson, 1976; Packard, 1978), the segmentation anomalies must have arisen by modification of the specified somite pattern under circumstances that did not allow restoration of the proper pattern. However, Menkes and coworkers (Menkes and Miclea, 1962; Menkes and Sandor, 1969) have shown most impressively that somite formation continues normally (after a brief delay) even when the organization of the segmental plate has been thoroughly disrupted by cutting the tissue, along with the adherent ectoderm and endoderm, into many pieces and mixing the fragments. Also, Sandor (1972) excised areas of future intersomitic furrows from the segmental plates of explanted chicken embryos and found that the somite pattern still formed normally. In these instances, unlike the heat-shock experiments, a mechanism for rapidly restoring the pattern must have functioned. Our experiments were designed to explore further the capacities of the embryo for modification and reconstitution of the segmental pattern. Some of these results have been published in an abbreviated form (Packard, 1986b).

MATERIALS AND METHODS

Fertile White Leghorn chicken eggs and fertile Japanese quail eggs were obtained from the Poultry Science Division of Cornell University, Ithaca, New York. The eggs were stored in a humidified atmosphere at 9°C until needed. After incubation at 38°C in an egg incubator for about 48 hours, each egg was opened into a bowl of Howard’s (1953) saline and the embryos were removed from their yolks, washed briefly in calcium- and magnesium-free Tyrode’s (1910) solution and then placed at room temperature in about 5 ml of calcium- and magnesium-free Tyrode’s solution containing 1% trypsin (hog pancreas, ICN Pharmaceuticals, Inc.) and 0.1% ethylenediaminetetraacetic acid (EDTA). The embryos remained in this solution for 5 to 15 minutes; the time varied with the embryo’s age and the amount of yolk present. When the embryo’s neural tube appeared wrinkled or open, the embryo was transferred to a 35 mm plastic culture plate containing a nutritive agar substrate and about 1 ml of fresh, chicken, whole-egg supernatant (Packard and Jacobson, 1976). The excess supernatant was drawn off and microsurgery was performed with electrolyti-cally sharpened tungsten wire needles. The ectoderm overlying the segmental plate was cut into a flap that was folded laterally to expose the segmental plate. The exposed segmental plate was carefully separated from the neural tube and surrounding mesoderm and then it was gently peeled from the underlying endoderm. The node region of the embryo, including all three germ layers, was completely removed to prevent continued formation of segmental plate mesoderm (Packard and Jacobson, 1976; Packard, 1980a,b). Grafting of segmental plates was accomplished by transferring the segmental plate to the host embryo’s dish with fine watchmaker’s forceps. The graft was then inserted into the host’s removal site in such a way as to maintain the graft’s original dorsal-ventral and cranial-caudal orientation and the ectodermal flap was then closed over the graft.

The embryos were cultured in vitro at 38°C in a humidified atmosphere of 95% O2 and 5% CO2 for 16 to 20 hours (Packard and Jacobson, 1976); the duration of the culture period was chosen on the basis of previous work showing that explants containing segmental plates complete somite formation within 15 hours and begin to lose somites after 20 hours (Packard and Jacobson, 1976). The embryos were then fixed in ethanol:acetic acid (4:1), dehydrated, embedded in paraffin, cut coronally into 10 µm serial sections, stained according to the Feulgen-Rosenbeck technique (1924), counterstained with fast green, and camera lucida tracings of the neural tube and somites were made from appropriate sections of each embryo. The tracings were digitized with an acoustic tablet and the orthostereoscopic reconstructions were created using custom software (Falen and Packard, 1982).

In some experiments, an impermeable barrier was placed either medial or lateral to the segmental plate. In these experiments, chicken embryos were isolated, treated with enzyme and placed in culture plates as described above. The right segmental plate of each embryo was removed, cut into 15 to 20 pieces and the pieces were randomly reinserted into the removal site. The ectodermal flap was then replaced. A piece of tantalum foil was then cut so that its length matched that of the segmental plate. The foil was held in the desired position (either medial or lateral to the segmental plate pieces) and its edge was forced down through all three germ layers of the embryo and into the agar substrate. Finally, the node region was removed and the embryo was cultured and fixed as described above. After fixation, the foil was gently removed so as to avoid damage to the embryo. The embryo was then processed for histological examination as described above, except that the sections, which contained no quail cells, were stained with hematoxylin and eosin. Control experiments to test for any toxic effects of the foil were performed by inserting a tantalum foil barrier between the neural tube and the undisturbed right segmental plate of embryos prepared for culture but not treated with enzyme.

RESULTS

A series of control experiments was performed to estimate the effect of the experimental manipulations on the number of somites formed by grafted segmental plates. Chicken or quail embryos with 8 to 21 pairs of somites were treated with trypsin, and the right segmental plate of each embryo was removed and then replaced immediately as indicated in Fig. 1. The embryos were then cultured, fixed and analyzed histologically. A typical result from one of these experiments is shown in Fig. 2. A total of 73 embryos operated on in this way survived the culture period. Of these embryos, 42 were damaged during processing or showed obvious evidence that all or part of the manipulated segmental plate had been lost. These embryos were not included in the following analysis. As expected, when the numbers of somites formed by the right and left segmental plates of each embryo were counted from the stereoscopic reconstructions, a high correlation between the figures was revealed (r=0.904, n=31, P<0.001, Fig. 3); no species- or
Somite pattern regulation

Age-related differences were noted. To facilitate comparison of results in this and other experiments, an 'index of inequality' was calculated by averaging for all experiments the absolute difference between the two sides in each embryo; the higher the value of the index, the greater was the average difference between the sides. In the case of the control experiments, the index (X ± s.d.) was 0.68 ± 0.91 somites. Since previous studies had shown that segmental plates removed simultaneously from the same embryo tended to form the same number of somites (Packard, 1980a, Packard and Meier, 1983) and since a similar result was found in these control experiments, it was concluded that the manipulation of the segmental plates resulted in at most only a small change in the number of somites formed by them.

The first experimental question asked was: can a host embryo alter the number of somites that a grafted segmental plate will form? Right segmental plates were removed from trypsinized quail embryos that possessed 5 to 23 pairs of somites. The segmental plates were then grafted in place of the host's right segmental plate into trypsinized host chicken embryos containing 9 to 24 pairs of somites (Figs 4, 5). Both donor and host embryos were cultured for about 18 hours, fixed, sectioned and reconstructed. Would the grafted segmental plates form the same number of somites as the donor embryo or would the host environment cause them to form a number of somites more similar to that formed by the host embryo?

Fig. 1. Diagrams of control experiments performed in both chicken and quail embryos. Embryos with 8 to 21 pairs of somites were treated with trypsin and the ectoderm layer was folded laterally from the underlying segmental plate mesoderm. The segmental plate was carefully removed, intact, from the embryo and then returned to its original site in its original orientation, after which the ectoderm was replaced. The entire region containing Hensen’s node and the primitive streak was removed and discarded in order to prevent further formation of segmental plate. The embryos were cultured for 16 to 20 hours, fixed and analyzed histologically. In each species, the number of somites formed on the unoperated and operated sides was almost identical.

Fig. 2. Orthostereoscopic reconstruction of a fixed chicken embryo from the series of control experiments in which the right segmental plate was removed and replaced. Somites formed by the right, manipulated, segmental plate are indicated by the bracket. The left segmental plate formed 9 somites, while the right segmental plate formed 8 somites. The 3 cranialmost pairs of somites (top) were already present at the time of microsurgery.

Fig. 3. Summary of results of 31 control experiments (15 chick and 16 quail). The number of somites formed by the right, manipulated, segmental plates was very similar to the number to the number formed by the left, unoperated, segmental plates (r=0.904, P<0.001). In this and other graphs, the line represents the calculated linear regression and coincident results are indicated by concentric rings.
mesoderm or quail cells on their operated sides. In these cases, the grafts had apparently been lost from the embryos.

Reconstructions from a typical experiment are shown in Fig. 6. This experiment differed from the previous control experiment only in that the excised segmental plate was grafted into another embryo rather than being returned to the donor. Therefore, the number of somites formed by the donor’s left, unoperated, segmental plate was compared first with the number of somites formed by the graft (Fig. 7).

Comparison of the data in Figs 3 and 7 shows that, while removing a segmental plate from an embryo and returning it to its original location resulted in little change in the number of somites that it subsequently formed, removal of a segmental plate from an embryo and placing it into another embryo significantly altered the number of somites that it would form. This point was confirmed by both the much lower correlation between the number of somites formed by the two donor segmental plates \(r=0.305, n=30, P<0.10\) and the significantly higher index of inequality for this comparison \((2.00 \pm 2.24 \text{ somites}, t=2.43, df=59, P<0.02)\) than in the control experiments.

In order to see if the host embryos had influenced the grafts to form numbers of somites more compatible with the host’s somite pattern, the number of somites formed by the host’s unoperated left segmental plate was compared with the number of somites formed by the graft. As shown in Fig. 8, the correlation between these numbers was significant \((r=0.666, n=30, P<0.001)\). It was concluded that the host embryos demonstrated a significant ability to alter the number of somites formed by the grafts.

This change of somite number was even more apparent when the data were viewed in such a way that the differences in the number of somites that the donor and host
embryos were originally specified to make could be taken into account. The algebraic difference between the number of somites required to maintain the symmetry of the host’s somite pattern (indicated by the number of somites formed on the host’s unoperated side) and the number of somites that the graft was originally specified to make (prospective fate; indicated by the number of somites formed by the donor’s unoperated side) was defined as the ‘disharmony’ between host and graft under those experimental conditions. This figure for disharmony was then compared with the ‘response’ of the graft to the experiment. Response was determined by subtracting the number of somites that the graft was originally specified to make (again indicated by the number of somites formed by the donor’s unoperated side) from the number of somites that the graft actually formed. For example, if the host’s unoperated segmental plate formed 12 somites and the donor’s unoperated segmental plate formed 8 somites, the disharmony between host and graft would be +4; that is, the graft would have to form 4 somites, in addition to those it was originally specified to form, in order to maintain the host’s somite pattern. If the graft actually formed 10 somites, the response would be +2; that is, it formed two additional somites. Fig. 9 illustrates the relationship between disharmony and the response of the graft. The correlation between these two measures was statistically significant ($r=0.755$, $n=30$, $P<0.001$), and although nine grafts failed to respond, only 4 of the 30 grafts responded in an unexpected direction; that is, when the disharmony in a given experiment was positive or negative, the response of the graft tended to be, respectively, positive or negative. While most of the grafts did respond, their response was imperfect; the number of somites that they made was often more than or less than the number one might have predicted from the disharmony.

The reason for this incomplete or imperfect response of the grafts to the host embryos is not clear. We wondered whether this phenomenon might be due to the fact that the cells in the cranial portion of the grafted segmental plate must have formed somites within a few hours after the surgery. It seemed possible that there was insufficient time for the host embryo to alter the number of somites formed by the cranial portion of the graft or that the pattern near the cranial end of the segmental plate was more stably specified than that in the more caudal parts of the segmental plate and hence not susceptible to host influence. To test this possibility, we performed experiments in which the caudal half of the right segmental plate was removed from two quail embryos. These two caudal segmental plate halves were then grafted together in place of the right segmental plate of a chicken embryo (Fig. 10). Thus, an amount of segmental plate mesoderm equivalent to one segmental plate was grafted, as in the previous experiments, but in this case all of the grafted mesoderm originated from the caudal halves of segmental plates. Would the number of somites formed by caudal segmental plate grafts more closely match the number of somites formed by the unoperated segmental plate of the host chicken embryo? Eight of the twenty experiments thus performed resulted in sections suitable for reconstructive analysis. At the time of surgery, the chicken embryo hosts possessed from 12 to 22 somite pairs, while the quail donor embryos possessed 12 to 23 somite pairs. The result of the experiment is seen

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**Fig. 8.** Graph comparing the number of somites formed by grafted, quail, right segmental plates with the number of somites formed by unoperated, left segmental plates of host chicken embryos. The correlation between these numbers was significant ($r=0.666$, $n=30$, $P<0.001$). This result indicates that the number of somites formed by grafted segmental plates more closely resembled the number of somites formed by the host embryo than the number formed by the donor embryo.

**Fig. 9.** Graph to compare the ‘disharmony’ between the number of somites the graft and host segmental plates were specified to form with the ‘response’ of the graft, in terms of whether it formed fewer or additional somites than it was originally specified to form (see text for further explanation). The grafts tended to form a number of somites more compatible with the host embryo somite pattern ($r=0.755$, $n=30$, $P<0.001$).

**Fig. 10.** Diagram of experiment in which the right segmental plates of chick embryos were replaced with two grafts, each consisting of the caudal half of a quail segmental plate. This experiment was designed to determine if caudal halves of segmental plates were better able to respond to the influences of the host embryos than intact segmental plates.
most clearly when the data are expressed as disharmony versus response, as in the previous experiment (Fig. 11). In this case, the value of the disharmony for each experiment was determined by subtracting the number of somites formed by the unoperated side of the host embryo from the total number of somites missing from the operated sides of the two donor embryos (the number of missing somites was judged by subtracting the number of somites formed on the right [operated] side from the number of somites formed on the left [unoperated] side). A response pattern very similar to that resulting from the grafting of intact segmental plates was observed. The correlation coefficient was 0.814, \( n=8, P<0.01 \). Thus, it is clear from these experiments that caudal segmental plate mesoderm is not more responsive to the influences of the host embryo.

It also seemed possible that the variable response of the grafts to the host environment might, in part, be due to the interaction between putative pattern-specifying signals emanating from the host and the specified somite (somitomere) pattern of the graft. Therefore, a fourth series of experiments was carried out in which the excised quail segmental plate was bisected lengthwise, cut into about 20 pieces (range=14 to 25 pieces), and the pieces randomly inserted into the host embryo (Fig. 12). These experiments were identical to the preceding intact segmental plate grafting experiments except that the specified somite pattern of each graft had been disrupted by cutting the grafts into pieces and scrambling the positions of the pieces. Segmental plates treated in this way will be referred to as having been ‘disrupted.’ It was asked whether the change in somite number would still occur and, if so, whether disruption of the graft’s specified somite pattern would permit the host embryo to regulate the grafted mesoderm to form a number of somites more similar to that formed by the host. A total of 39 such experiments were performed. The embryos in 17 of these experiments survived the culture period and processing for histological study and were judged suitable for analysis. A few experiments were excluded because there was either no somite mesoderm or obvious gaps in the somite mesoderm on the host embryo’s operated side. In these cases, some or all of the graft segmental plate mesoderm had apparently been lost. When the numbers of somites formed by the donor embryos were compared with the numbers of somites formed by the disrupted and grafted quail segmental plates (Fig. 13), it was evident that there was no significant correlation between these figures (\( r=0.182, n=17, P<0.72 \)). In order to see whether the grafts had formed numbers of somites more similar to the numbers formed by the hosts, the number of somites formed by the disrupted quail segmental plate grafts was compared with the number of somites formed by the host chick embryos (Fig. 14). There was a weak correlation between these numbers (\( r=0.400, n=17, P<0.03 \)). However, once again, when these same data were viewed with respect to the previously described values of disharmony and response for each experiment (Fig. 15), it was clearly evident that a significant relationship between these variables was present (\( r=0.850, n=17, P<0.001 \)). Thus, the disruption of the graft’s segmental pattern did not appear to affect significantly the graft’s responsiveness to the host’s influences.

The orthostereoscopic reconstructions used in this study not only aided in the accurate counting of somites, they also
made it possible to compare the somite patterns observed in the experiments. Analysis of the numbers of somites formed in the above experiments suggested that the grafted segmental plates responded to the host embryo’s environment by tending to form a number of somites different from the number of somites that they were originally specified to form. Study of the tissue reconstructions also suggested that there was a tendency for the positions of the intersomitic interfaces between the graft-derived somites to occur at nearly the same axial level in the host embryo as the intersomitic interfaces of the host’s segmental pattern. For example, when the cranial-to-caudal lengths of the somites formed by the donor embryo were much longer or shorter than those formed by the host embryo, the somites formed by the graft tended to be of a size that was appropriate for the host’s segmental pattern. An example of this phenomenon is shown in Fig. 16. Fig. 17 shows another interesting example of this phenomenon. This figure is a reconstruction of a chick host embryo fixed 18 hours after the transfer of a disrupted quail segmental plate graft. The host’s unoperated left segmental plate formed 4 somites. The donor embryo (not shown) formed 7 somites on its unoperated side and the graft also formed 7 somites (4 pairs of host somites are shown cranial to the cranial extent of the graft). In terms of somite number, therefore, it would appear that the graft did not respond. However, close inspection of the reconstruction suggests that somite sizes and locations were adjusted to place a graft intersomitic interface at the axial level of each host intersomitic interface. The 4 most cranial pairs of somites (toward top) were present at the time of microsurgery. Quail somites in the host embryo are marked with small dashes.

occupied almost the same position in the pattern as the missing host somite would have occupied. The graft formed 1 somite opposite the host’s second somite and 3 small somites opposite the host’s third somite. Thus, while there was no change in the number of graft somites, there was an apparent adjustment of the length and location of the graft somites in such a way as to preserve better the symmetry of the host’s somite pattern. Although such adjustment was not always as clear as in this example, we judged it to be demonstrated at least to some extent in 21 of the 30 intact graft experiments. Therefore, the host embryos may have a more extensive ability to alter the graft somite pattern than was suggested by the study of somite numbers alone.

The demonstration that host embryos can alter the somite pattern of grafted segmental plates raises the question of...
what regions of the host embryos are active in this alteration. We decided to test whether tissues either medial or lateral to the segmental plate are important to the reestablishment of the somite pattern in disrupted segmental plates. Impermeable tantalum foil barriers were inserted either medial or lateral to the pieces of a disrupted segmental plate and, after the same culture period used in the previous experiments, the resulting somite pattern was observed.

First, a control experiment was performed to test for any possible toxic effects of the tantalum foil barrier. Four chicken embryos having 11 to 19 pairs of somites were placed on the agar culture substratum and a piece of foil, equivalent in length to the segmental plate, was placed between the neural tube and the right segmental plate. The foil was pushed entirely through the embryo and into the agar (Fig. 18). After 16 to 20 hours in culture, each of the embryos had formed symmetrical somite pairs through the level of the foil (Fig. 19). The foil did not appear to interfere with somite formation.

In the second series of experiments, chicken embryos possessing approximately 10 to 20 somite pairs were treated with trypsin, placed on the agar culture medium, and their right segmental plates were removed. The segmental plates were then cut into 15 to 20 pieces and the pieces were randomly reinserted into the removal site. The tantalum foil barrier was then placed immediately lateral to the pieces (Fig. 20). Following the culture period, the embryos were fixed, the foil was removed and the embryos were processed for histological study. Seven embryos were judged suitable for study. In two of the experiments, there was no somite mesoderm on the embryo’s right side (the side with the foil barrier). Apparently, the pieces of segmental plate had been lost during the culture period. Somites that appeared to be normal were observed medial to the barrier in the remaining five experiments (Fig. 21). However, in each of the experiments, there were fewer somites on the operated side (Table 1). The smaller number of somites on the operated side was always associated with one or more obvious gaps in the row of somites. This observation suggested that one or more of the segmental plate pieces had been lost.

In the final series of barrier experiments, chicken embryos were treated exactly as for the previous experiments, except that the foil barrier was placed between the neural tube and the disrupted segmental plate (Fig. 22). Sixteen embryos survived the culture period and histological processing. The mean number of somites formed on the
unoperated side of these embryos was 8.5, while the mean number formed on the operated side was 1.1 (Table 2). Nine of the sixteen embryos formed no somites on the side with the barrier and the remaining embryos formed fewer somites on that side than on the unoperated side. A dense mass of unsegmented paraxial mesoderm was always present on the operated side. Gaps in this mesoderm suggested that some of the grafted pieces had been lost (see text).

Table 2. Results of experiments in which tantalum foil barriers were placed medial to disrupted segmental plate

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*Nine of the sixteen embryos formed no somites on the side with the barrier and the remaining embryos formed fewer somites on that side than on the unoperated side. A dense mass of unsegmented paraxial mesoderm was always present on the operated side. Gaps in this mesoderm suggested that some of the grafted pieces had been lost (see text).
myocoels with a very short somite epithelium so that the 

\[ \mu \text{m} \]

taking on the appearance of hollow vesicles. It was 

vertical through the center of the figure is the space that was 

clear that, once the prepattern of segmentation in the seg-

mental plate had been destroyed by ‘disrupting’ the seg-

mental pattern can either be modified by heat shock without 

restoration of the original pattern or reestablished follow-

ing surgical disruption. By taking advantage of the known 

bilateral symmetry of the segmental pattern in the two seg-

mental plates of any given embryo, we were able to devise 

experiments that revealed the ability of the avian embryo 

to modify the segmental pattern.

The control experiments showed that the surgical manip-

ulations required for segmental plate grafting did not sig-

ificantly alter the number of somites formed by a seg-

mental plate. But because the correlation between the numbers 
of somites formed on the unoperated and operated sides of 

these embryos was so high, we expected to be able to detect 

a change of two or more in the number of somites formed 

by segmental plates grafted homotopically. It is interesting to 

to compare these control data with those published in figure 8 of Packard (1980a). At that time, both segmental plates 

were removed from Japanese quail embryos as separate 
tissue explants that included the lateral plate mesoderm, 
ectoderm and endoderm, along with various combinations 
of neural tube and notochord. The explants were cultured 

for 14 to 20 hours and the number of somites formed in 

the left explant from each embryo was compared with the 

number formed in the right explant. The correlation 

between these numbers was significant \((r=0.657; P<0.001; 
\text{n}=90)\), and was taken to indicate that segmental plates 

removed at the same time from the same embryo form 

nearly the same number of somites. The major difference 

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graft experiments. The only explanation we have for these 

low somite counts is that the cuts made at the caudal end of 

the segmental plate must have tended to be more cranial 
than similar cuts made about ten years earlier. This would 
have led to smaller numbers of somites while maintaining 
the bilateral symmetry. It is important to note that while 
the mean somite number was lower in the recent series, the 
slope of the linear regression is remarkably similar in both 
sets of data, which suggests that the left-right symmetry in 
the two groups of experiments was the same. Therefore, the 
enzyme treatments and surgical manipulations used in the 

present experiments did not introduce dissimilarity between 

the operated and unoperated segmental plates.

There is compelling evidence that at least some of the 
cells along the entire length of the avian segmental plate 
are committed to forming a particular somite pattern. (1) 
Segmental plates from avian embryos are able to form 
somites in the absence of further contact with the tissues 
that normally surround them (Sandor and Amels, 1970; 
1971; Packard and Jacobson, 1976; Bellairs and Veini, 
1980; Sandor and Fazakas-Todea, 1980). In addition, when 
avian segmental plates are cultured, each forms somites 
along its entire length. These somites are usually 10 to 12 
in number (Packard and Jacobson, 1976; Packard, 1978; 
1980a). (2) Although there is some variation in the number 
of somites formed by segmental plates removed from dif-

DISCUSSION

The work presented here was designed to investigate the 
related but distinct capacities of the segmental plate to 
undergo modification or restoration of the segmental pat-
tern. As described in the Introduction, we were intrigued 
by the apparent contradiction between evidence indicating 
that the segmental pattern is already firmly established 
within the segmental plate and evidence indicating that the 
pattern can either be modified by heat shock without 
restoration of the original pattern or reestablished follow-
ing surgical disruption. By taking advantage of the known 
bilateral symmetry of the segmental pattern in the two seg-

mental plates of any given embryo, we were able to devise 
experiments that revealed the ability of the avian embryo 
to modify the segmental pattern.

The control experiments showed that the surgical manip-

ulations required for segmental plate grafting did not sig-

ificantly alter the number of somites formed by a seg-

mental plate. Because the correlation between the numbers 
of somites formed on the unoperated and operated sides of 

these embryos was so high, we expected to be able to detect 

a change of two or more in the number of somites formed 

by segmental plates grafted homotopically. It is interesting to 
to compare these control data with those published in figure 8 of Packard (1980a). At that time, both segmental plates 

were removed from Japanese quail embryos as separate 
tissue explants that included the lateral plate mesoderm, 
ectoderm and endoderm, along with various combinations 
of neural tube and notochord. The explants were cultured 

for 14 to 20 hours and the number of somites formed in 

the left explant from each embryo was compared with the 

number formed in the right explant. The correlation 

between these numbers was significant \((r=0.657; P<0.001; 
\text{n}=90)\), and was taken to indicate that segmental plates 

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ferent embryos, when both the right and left segmental plates are removed from a given embryo, they are very likely to form the same or nearly the same number of somites after a suitable time in culture (Packard, 1980a; 1986b). (3) If, during early somitogenesis, the node, primitive streak and the immediately adjacent region of the embryo are removed, the embryo will produce about 10 additional pairs of somites and then somite formation stops (Packard and Jacobson, 1976). Removal of the node and streak apparently halts the formation of new segmental plate, so that somite formation continues only until the somite prepattern present in the segmental plates at the time of the operation is expressed. (4) When segmental plates are transected, each resulting piece is able to form the portion of the somite pattern that it would have formed if the cuts had not been made, since the total number of somites made by the pieces is still 10 to 12 (Packard, 1978). Similarly, when segmental plates are bisected lengthwise, they form double files of small somites, with the reduplicated somites usually being of equal number (Menkes and Miclea, 1962). (5) When the segmental plate is excised and reimplanted so that the cranial-caudal axis is reversed, it forms somites in a caudal-to-cranial sequence, that is, in the sequence consistent with its original orientation (Menkes et al., 1968; Menkes and Sandler, 1969; Christ et al., 1974). (6) When a segmental plate is observed with stereo scanning electron microscopy, about 10 ‘somitomeres’ can be seen arranged in tandem along its entire length (Meier, 1979, 1982a,b; Tripplett and Meier, 1982). These regions of cells, with their processes arranged in a circular pattern, have been shown to be present in equal numbers in the two segmental plates of a given embryo (Packard and Meier, 1983). Furthermore, in explanted segmental plates, somite formation proceeds at the expense of preexisting somitomeres; that is, somitomeres normally become somites (Packard and Meier, 1983).

Nevertheless, when a segmental plate was placed in another embryo, rather than returned to its host embryo as in the control experiments, the number of somites formed by the graft was significantly different from the number formed by the unoperated segmental plate of the donor. Does such evidence that a segmental plate can be influenced to make more or fewer somites than it was originally specified to make conflict with the evidence that the avian segmental plate contains a prepattern of somite segmenta- tion? We would expect that altering the somite pattern would require that the specified segmental pattern and, therefore, the somitomeric prepattern be changed. Whether such a respecification of the prepattern seems plausible depends on how one conceives of somitomeres. One view is that somitomeres are stable, rather rigid structures, as exemplified by Keynes and Stern (1988): “in order to maintain a fixed arrangement of somitomeres, it is essential that there be little or no cell movement within the segmental plate, or at least that any movement be restricted to a single somitomere.” For these authors, somitomeres seem to be the formal equivalent of somites. If one insists on this view, it is indeed difficult to imagine how somitomeres might be created or destroyed in adjusting the prepattern.

The alternative view, which we favor, is to regard somitomeres as dynamic structures, recognizable by patterns of cell orientation within a layer of mesodermal cells that is constantly changing because of cell division and cell movements (Jacobson and Meier, 1986; Jacobson, 1988; 1992). Anderson and Meier (1981) have shown that, as the chick cranial neural crest migrates over the dorsal surface of the head paraxial mesoderm, the cells of the neural crest take on the characteristic swirling pattern of the underlying mesodermal somitomeres. So, even a sheet of migrating cells can exhibit the somitomere pattern. If these patterns of cell orientation are so readily influenced as to permit a moving sheet of neural crest cells to take on the underlying somitomere pattern, one can imagine that the cells of a grafted segmental plate could adjust their cellular orientations in response to host influences to reflect more closely the original segmental pattern of a host embryo. Recalling that the somitomere pattern ultimately becomes transformed into the somite pattern (Packard and Meier, 1983), we suggest that the putative adjustment of the somitomere pattern would reflect a respecification of the segmental pattern which, in turn, would lead to the altered numbers of somites in our grafted segmental plates. In any case, it is clear that, to use the terminology of Slack (1991, pp. 18-33), the specified segmental pattern in the avian segmental plate is not determined, but is capable of regulation when the segmental plate is grafted homotopically into another embryo.

The numerical evidence for the response of grafts to the host embryo environment was confirmed and amplified by analysis of the orthostereoscopic reconstructions. It was apparent from comparing the somites formed in the grafts with those in the donor and host embryos that the location and size of somites could be adjusted by the host embryo. The size of the graft somites tended to be similar to that of the host somites. When the graft made more somites than the host, two to three small somites were often seen in the space normally occupied by one somite. The impression that we gained from studying the reconstructions was that an intersomitic interface tended to form on the operated side opposite each intersomitic interface on the unoperated side, while additional intersomitic interfaces on the operated side could occur at any level. Even the occasional graft that showed no response at all in terms of the number of somites it formed could show clear evidence of a response in the reconstructions. Thus, the ability of the grafts to respond to the influences of the host was even more pronounced than was suggested by the numerical evidence alone.

What components of the host embryos bring about or influence the respecification of the graft segmental pattern? We attempted to address this question by placing an impermeable barrier either medial or lateral to disrupted segmental plates. While somite formation was unimpeded when the barrier was lateral to the pieces of segmental plate, at most only a few somites formed when the barrier was placed medial to the grafted fragments. We considered the possibility that when barriers were placed medial to the disrupted segmental plates, blood from the dorsal aorta was unable to reach the segmental plate cells. Impaired blood supply might account for the failure of somite formation. However, when barriers were placed medial to the segmental plates in unoperated chick embryos, somites formed in a normal fashion. These results, therefore, suggest that contact with tissues near the
midline of the embryo is required for disrupted segmental plates to form somites.

In view of the results of the barrier experiments, could it be that the neural tube and/or the notochord are segmented at the level of the segmental plate? If so, these structures might guide the reconstitution of the segmental pattern in the adjacent segmental plate mesoderm. Stern (1990) has suggested that the avian notochord is the archetypal segmented structure in the trunk of vertebrates and that it may imprint segmental information onto the adjacent paraxial mesoderm. Kimmel et al. (1991) argue strongly against this notion, noting that zebrafish lacking a notochord are segmented. However, even if the paraxial mesoderm can segment in the absence of notochord, it remains possible that axial position is similarly specified in notochord and segmental plate and that this positional information is used to maintain the axial and paraxial tissues in register. With regard to the neural tube, the evidence available at this time suggests that it does not acquire segmental properties until after somite formation in the adjacent mesoderm (Kimmel et al., 1991; Stern et al., 1988, 1991). Indeed, Stern et al. (1991) have shown that segmental lineage restrictions in the chick neural tube are dependent on the presence of somites. Despite the lack of evidence for segmentation of the neural tube and notochord at the level of the segmental plates, it is possible that the presence of the somitomere pattern in the segmental plate could lead to a segmental variation in some molecular entity (e.g., a component of the extracellular matrix) distributed along the sides of the axial structures. Following experimental disruption of the somitomere pattern, these segmental variations might guide the restoration of the segmental pattern.

Our results, then, suggest a possible interaction between axial structures and the presomitic mesoderm in maintaining the symmetry of the segmental pattern. It is important to distinguish this proposed interaction from two other types of postulated interaction between axial tissues and paraxial mesoderm. The first of these interactions, whereby axial level is specified within the paraxial mesoderm, is thought to occur earlier during gastrulation. Kessel and Gruss (1991), who analyzed homeotic transformations of vertebral cDNA in mouse embryos treated with retinoic acid during gastrulation, propose the following mechanism for specification of the embryonic axis: “A retinoic acid signal generated from midline embryonic structures during gastrulation is received by ingressing cells, which respond with the sequential activation of more and more Hox genes,” thus defining more and more caudal levels along the embryonic axis. While it is conceivable that retinoic acid from Hensen’s node participates somehow in establishing the somitic prepattern at the same time that it activates Hox genes, the evidence summarized above suggests instead that elements of the segmental prepattern form caudal to Hensen’s node by an independent mechanism and that it is these elements (recognizable as somitomeres) that are receiving and responding to the putative retinoic acid signal.

It would be of great interest to examine vertebral structures and patterns of Hox gene expression after disruption of segmental plates as described in this paper; it may be that, even though the presomitic pattern is seemingly restored, proper axial specification is not. The second axial-paraxial interaction may occur at later stages, just before or shortly after somites have formed, and probably plays an important role in the formation of the sclerotome and dermamyotome (Kenny-Mobbs and Thorogood, 1987; Aoyama and Asamoto, 1988), shaping of the somites (Packard and Jacobson, 1979; Drake et al., 1992) and the further development of the sclerotome after its formation (Packard and Jacobson, 1976; Drake et al., 1992). In the absence of other evidence, this later influence on somite differentiation must be presumed distinct from the proposed function of axial structures in maintaining the symmetry of the somitic prepattern.

We believe that the results of our experiments, when considered along with the literature cited above, are most plausibly explained as follows. Although the segmental plate contains a specified segmental pattern, this pattern is not determined and so is subject to regulation. When a segmental plate is grafted homotopically into a host embryo, the cells in the graft change their positions and reorient their cell processes in response to influences emanating from tissues near the midline of the host. This reorientation leads to a new specified segmental pattern in the form of a new somitomere pattern, that subsequently is expressed as an altered somite pattern. It is conceivable that pattern disruptions induced by heat shock and other agents (Primett et al., 1988, 1989) are often repaired by such respecification so that only the most severe damage is later detected in the somite pattern. Further study of heat-shocked embryos may be very helpful in understanding somite pattern regulation.

It is not clear whether the pattern-regulating influences demonstrated here represent a continuation of the processes that originally established the segmental pattern in the segmental plate or whether they represent a separate mechanism that maintains the specified pattern until it is expressed as somites. The presence of a bilaterally symmetrical segmental pattern has great importance in the evolutionary sense, because disruptions or incompatibilities in that pattern would lead to problems in locomotion. The continuation of pattern-specifying processes or the presence of a corrective process to repair disturbances of the specified segmental pattern would help ensure a symmetrical somite pattern.

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