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A hierarchy of determining factors controls motoneuron innervation

Experimental studies on the development of the plantaris muscle (PL) in avian chimeras*

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Summary. Quail leg buds were grafted in place of chick leg buds or chick wing buds and vice versa at stages 18 to 21 after colonization by muscle precursor cells had been completed. Motor endplate pattern in the plantaris muscle of the grafts was analyzed before hatching by means of esterase and acetylcholinesterase staining techniques. Muscle fibre types were made visual using the myosin ATPase reaction. Investigations are based on the species-specific endplate pattern of the plantaris muscle: multiply innervated fibres in the chick and focally innervated fibres in the quail. Muscle pieces isolated from the adjacent medial gastrocnemius muscle of the grafted legs were histologically examined to judge their species-specific composition. Horseradish peroxidase was injected into the plantaris muscles of both the grafted and the opposite leg as well as in the plantaris muscle of normal quail embryos, in order to be sure that the plantaris muscle of the graft is innervated by appropriate motoneurons. This procedural design offers for the first time a possibility to test experimentally the influences of motoneurons on endplate pattern formation under conditions corresponding to those in normal ontogenesis. It is shown that such appropriate motoneurons of one species which project to the plantaris muscle of the other species dictate the endplate pattern. When the plantaris muscle is innervated by inappropriate motoneurons, the endplate pattern inherent in the muscle primordium itself becomes realized. A sequence of hierarchically acting factors is proposed to bring different results in line. According to this, the neuronally set programme has priority compared with that set in the muscle. This is true for the normal development and might generate the high neuro-muscular specificity. If under experimental conditions the neuronal programme and the peripheral programme differ, the axons and muscle fibres selectively interact with respect to their inherent characteristics and the muscle-specific programme becomes expressed. If there is a lack of a certain axon type, muscle fibres might become innervated by non-corresponding motoneurons which alter the muscle fibre type.

Key words: Muscle innervation – Endplate pattern formation – Hierarchy of controlling factors – Muscle fibre types – Plantaris muscle – Quail-chick chimeras

Introduction

Limb muscles of birds are found to be made up of focally innervated fibres, multiply innervated fibres, and a mixture of both types (Ginsborg 1960; Hess 1961; Barnard et al. 1982). The posterior latissimus dorsi muscle (PLD), for example, is composed of focally innervated fibres that are known to contract fast. The anterior latissimus dorsi muscle (ALD), on the other hand, contains multiply innervated fibres which are slow. Most of the limb muscles are formed by focally innervated fibres. Besides the ALD, the dorsal part of the ulnimetacarpalis dorsalis muscle (UMD) is formed by multiply innervated fibres whereas the ventral part of this muscle is focally innervated (Grim et al. 1983). Another well known chick muscle exhibiting mainly multiply innervated fibres is the adductor profundus muscle (Barnard et al. 1982).

The innervation type of the plantaris muscle (PL) has recently been studied in detail (Grim et al. 1985). Continuing former observations of Melichna et al. (1974) who found the PL to be mainly multiply innervated, Grim et al. compared the innervation types of the chick PL and the quail PL. They discovered that, unlike the multiply innervated chicken PL, the PL of the quail shows only one or two bands of endplates on the surface. The majority of isolated muscle fibres is found to be focally innervated by "en plaque" endplates. On the basis of this species-specific endplate pattern the PL can be used as a model suited to study the motoneuron-muscle interactions in developing chimeras.

Regarding the development of functional motor endplates several steps can be distinguished. It is widely accepted that axons growth on stereotyped highways into the limb bud and the relation between motoneurons and muscle cells display a high specificity (Hollyday 1980; Landmesser 1980). According to the concept of motoneuron-muscle specifity, motoneurons are initially determined with respect to their peripheral destination (Landmesser and Morris 1975). On the other hand, experimental studies have given

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Abbreviations: *PL*, plantaris muscle; *PLD*, posterior latissimus dorsi muscle; *ALD*, anterior latissimus dorsi muscle; *UMD*, ulnimetacarpalis dorsalis muscle; *E*, days of embryonic development; *HRP*, horseradish peroxidase; *LMC*, lateral motor column; *LS*, lumbosacral

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evidence that also a mismatch can occur of motoneurons are forced to innervate a foreign muscle (Bennett and Pettigrew 1974; Jacob et al. 1983; Jirmanová and Zelená 1973).

Where are the factors situated which determine the endplate pattern of limb muscles? At first glance, there appear to be two possibilities: the motoneurons determine the synaptic pattern and fibre type distribution, or the developing muscle itself controls this process. Observations exist that support both points of view. On the one hand, heterotopically replacements of pieces of the neural tube, for instance, as were done by Khaskiye et al. (1980), seem to prove the hypothesis favouring the determining influence of motorneurons. On the other hand, Jacob et al. (1983) have shown that an UMD inappropriately innervated does not change its normal endplate pattern. Additionally, Butler et al. (1982), as well as Phillips and Bennett (1984), show the initial differentiation of distinct fibre types analyzed by myosin-ATPase profiles appears normally even in aneurogenic limbs.

Since it is common to most of the experimental approaches being aimed at this problem that muscles are always forced to interact with inappropriate motoneurons, the question remains whether the results obtained are true for normal neuro-muscular interactions occuring during normal ontogenesis. The species-specific differences of the endplate pattern in the PL of chick and quail thus represent a favourable model to study interactions between muscle fibres and neurons of corresponding characteristics.

In this paper we focus on the PL in quail-chick chimeras addressing special attention to the formation of endplate pattern of PL muscles in the quail leg connected with appropriate or inappropriate motoneurons of the chick spinal cord and vice versa. Therefore leg buds of one species have been grafted in the place of leg or wing buds of the other species. A hierarchy of controlling factors is proposed which come from motoneurons as well as muscle primordia.

Materials and methods

Eggs of White Leghorn chick (Gallus domesticus) and of Japanese quail (Coturnix coturnix japonica) were incubated at 38° C and 80% humidity. Young embryos were staged according to the criteria of Hamburger and Hamilton (1951) ("HH-stages"), older embryos corresponding to the total period of incubation (e.g. E 18 = 18 days). After opening of the egg containing the host, the embryo was floated up to the level of the shell window by dripping Locke solution (Hara 1971). For microsurgical procedures iridectomy scissors and electrolytically sharpened tungsten needles (Dossel 1958) were used.

In a first series of experiments leg buds of quail embryos (HH-stages 18 to 21) were isolated and grafted in place of chick leg buds previously removed from embryos at corresponding stages. Grafts were fixed by the tension of the clipped amnion. In a second series of experiments quail leg buds were replaced by chick leg buds. Host and donor embryos ranged from HH-stages 18 to 21. In a third experimental series chick (or quail) leg buds were heterotopically transplanted in place of quail (or chick) wing buds. In these cases leg buds were temporarily fixed by means of cactus needles. The migration of somite-derived muscle precursor cells into the leg bud had been completed by the time of grafting (Jacob et al. 1979). Stages of embryos correspond to that used in the other series. E 16 to E 20 chick hosts

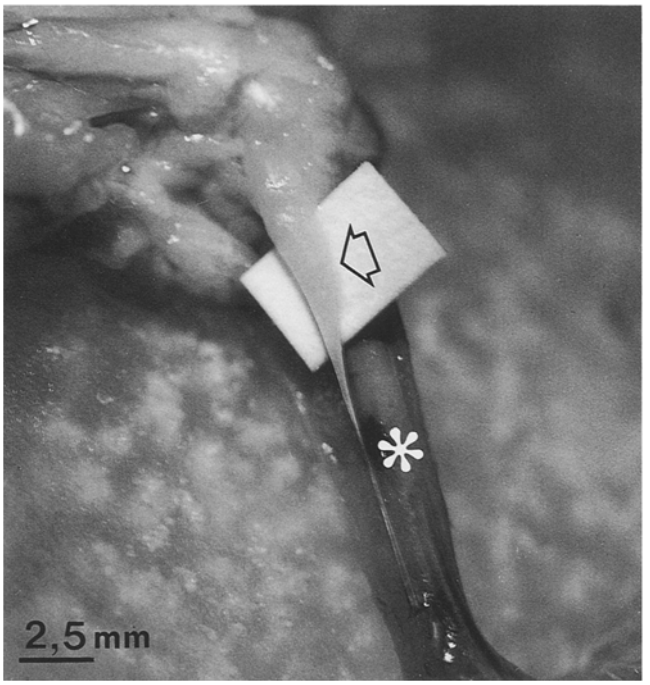


Fig. 1. Sectioned PL muscle of a 20-day chick embryo. *Arrow*: muscle belly; *asterisk*: tibia. Note the long tendon of the muscle

and E 15 or E 16 quail hosts were fixed respectively in 4% neutral formaldehyde and Serra's fluid. A total of 557 grafting experiments was performed and 96 embryos were evaluated. Only normally developed legs were used for evaluation. Limbs were skinned and a piece of muscle tissue from the proximal part of the medial gastrocnemius muscle, bordering on the PL, was used for histological examination. After microdissection the PL was fixed in situ for approximately 15 min. The isolated PL was further fixed for a total period of 1 to several hours. After washing in water the whole muscle was incubated at 37° C according to the indigogenic method of Holt (1958), as modified by Lojda et al. (1979), for demonstration of motor endplates. For comparison of endplate pattern the PL of the contralateral leg was treated in the same manner. In some cases endplates were demonstrated in teased small bundles of muscle fibres using the method of Karnovsky and Roots (1964). According to Barnard et al. (1982), and Grim et al. (1985), the chick PL is made up only by multiply innervated fibres. Therefore, the following criteria for classification of quail PL were used: 1. location of "en plaque" endplates at the dorsal PL surface in a limited zone, 2. fibres of a teased small bundles exhibiting only one endplate over a long distance (minimum: 2 mm).

PL and medial gastrocnemius muscle have the same origin. To make sure that the PL is formed by donor cells a piece of the adjacent gastrocnemius muscle was stained according to the method of Feulgen and Rossenbeck (1924). As the interphase nuclei of Japanese quail cells are characterized by a large mass of nucleolus-associated heterochromatin DNA which does not exist in the chick nuclei, quail cells can be distinguished from chick cells (Le Douarin 1969).

The qualified statement of Tanaka and Landmesser (1986) which concerns difficulties of distinction between quail and chick nuclei at late embryonic stages is not very

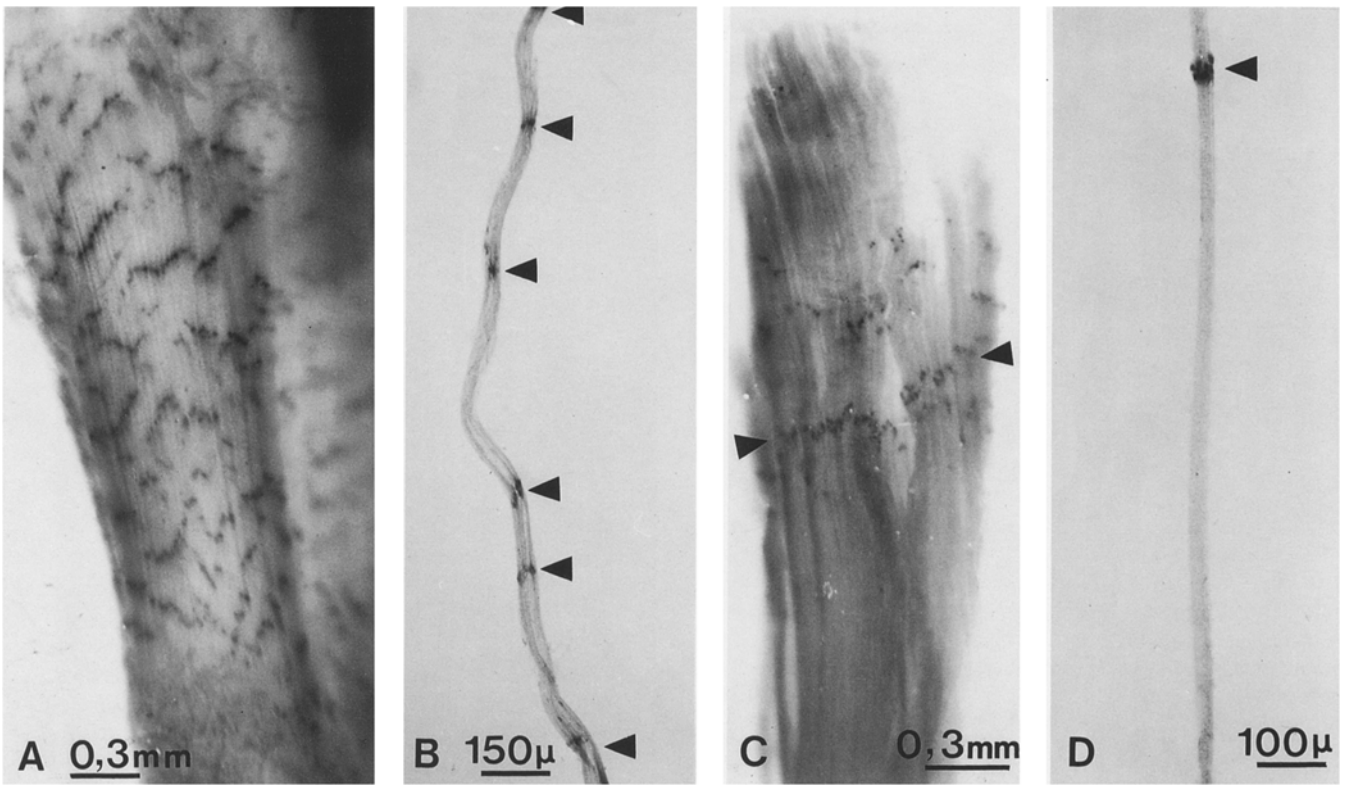


Fig. 2A–D. Normal PL muscles and muscle fibre bundles of chick and quail cut out from prehatching embryos. **A** Dorsal surface of a chick PL. Endplates are stained according to the indigogenic method. **B** Small bundle of a chick PL stained for acetylcholinesterase. Note the multiple innervation. **C** Dorsal surface of a quail PL. **D** Small bundle of a quail PL. Note the focal innervation. *Arrows*: endplates

well founded. Jacob et al. (1986) have shown that in muscles of chimeras 2 weeks after hatching nuclei can easily be distinguished. This seems to be a problem of fixation.

Fibre type composition of quail PL, chick PL and PL of chimeras was visualized using myosin ATPase reaction. Muscles were frozen in Freon cooled by liquid nitrogen. Unfixed cryostat sections (10 µm) were used for detection of myosin ATPase by the calcium-cobalt method of Padykula and Herman (1955) as modified by Lojda et al. (1979). Some sections were preincubated for 15 min by 20° C using potassium acetate buffer pH 4.3 according to Guth and Samaha (1970).

To prove that the PL in the grafted leg is appropriately innervated, the HRP method was used to demonstrate the motoneuron pool. E 10 and E 11 chick hosts were decapitated, eviscerated and skinned. The caudal body part was placed in Tyrode solution and PL of both legs was visualized by removing the medial part of the gastrocnemius muscle. HRP was injected through a glass pipette into the PL muscles of both the grafted and the opposite leg. In the same way, PL muscles of normal quail embryos (E 9 and E 10) were injected. Embryos were transferred into oxygenated Tyrode solution. Lumbosacral spinal cord was bared by removing the vertebral bodies. Specimens were incubated for 6 h at 32° C, fixed in glutaraldehyde for 1 h, washed for 6 days in TRIS-buffer changed several times and processed with diaminobenzidine (DAB) for development of the label. See for details of this procedure Landmesser (1978) as well as Tosney and Landmesser (1986). Specimens were dehydrated, embedded in paraffin, and transversally sectioned. The sections were counter-stained with cresyl violet.

Results

General

A normal chick PL prepared by sectioning can be seen in Fig. 1. Figure 2 shows the chicken PL and the quail PL which can be distinguished by species-specific patterns of motor endplates. While the chicken PL is multiply innervated the quail PL is focally innervated. The experimental procedures performed are based on the purpose of getting connections between the PL of one species with motoneurons of the other species. If a leg bud of one species is grafted in place of a leg bud of the other species motoneurons are allowed to find an appropriate target, as the scheme shown in Fig. 3 exemplary demonstrates. Results of these experiments are described in the first and second parts of this chapter. In the third part results are described which were obtained after replacement of wing by leg buds. According to the latter procedural design inappropriate motoneurons are forced to innervate the PL. Finally, the pools of motoneurons innervating the PL in normal and grafted legs are described.

I. Quail PL innervated by appropriate motoneurons of the chick

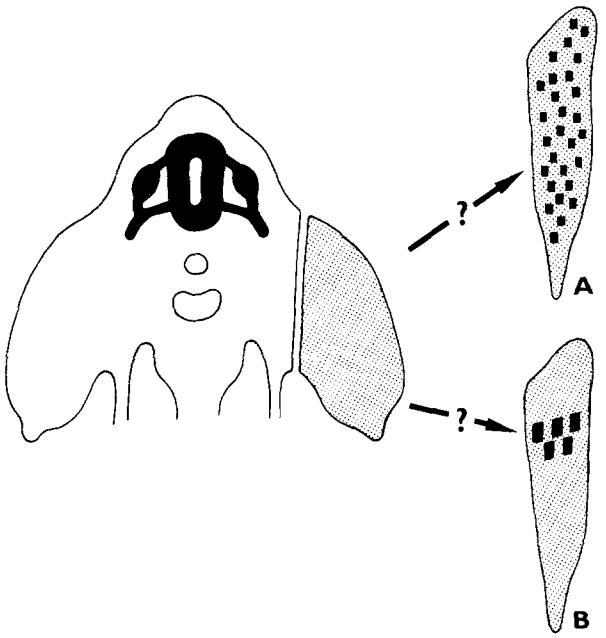


Fig. 3. Schematic representation of experimental procedures. Leg buds of one species are grafted instead of leg (or wing) buds in the other species. The question is whether the endplates of the PL innervated by motoneurons of the host neural tube develop in a chick-typical (*A*) or in a quail-typical (*B*) pattern. In the case illustrated, a quail leg bud (*grey*) is grafted in place of the right leg bud of a chick host (white). Black: neural tube spinal nerves and endplates of PL

Fig. 4. Chick host after grafting a quail leg (*arrow*) in place of the right chick leg and a subsequent reincubation period of 17 days

After grafting a quail leg bud instead of the chicken leg bud, in about 15% of the cases, a normally shaped quail leg was observed. Compared with the chick host leg of the contralateral side, its size is found to be in accordance with a normal quail leg at the corresponding developmental stage (Fig. 4). Depending on the age of the donor embryo at the time of grafting a more or less distinct feather pigmentation of quail characteristic can be seen. The skeletal and muscular pattern does not considerably differ from a normal quail leg.

The experimental design is based on condition that motoneurons of chick origin match muscle fibres of quail origin. To be sure about that, muscles of the grafts were histologically examined. Figure 5 shows an isolated piece cut out of the medial gastrocnemius muscle immediately covering the PL. The muscle fibres contain quail nuclei. Figure 6 shows the endplate pattern of quail PL innervated by appropriate chicken motoneurons. In more than 90% of evaluated specimen the endplate pattern is chick-typical, that is to say, the chick motoneurons must have determined and therefore "changed" the innervation type of the quail PL.

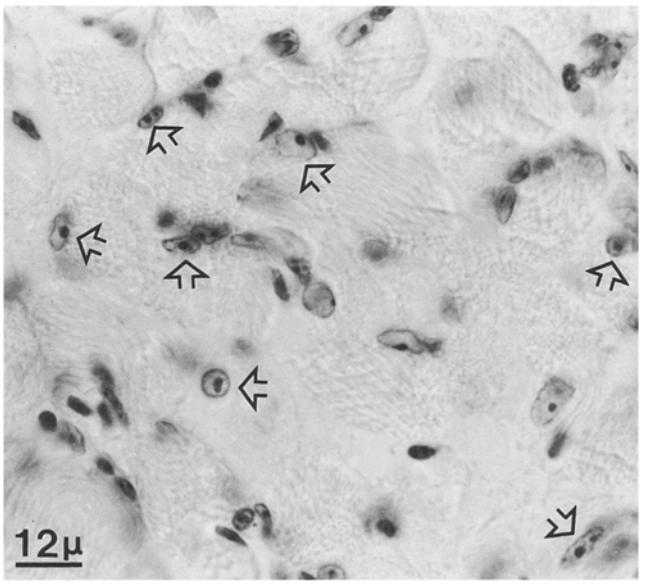


Fig. 5. Medial gastrocnemius muscle cut out from a grafted leg. *Arrows*: quail nuclei. Feulgen-staining

II. Chicken PL innervated by appropriate motoneurons of the quail

Results of this experimental series are similar to those obtained in the first one. Figure 7 shows a chick graft grown in place of a quail leg. While the quail host reveals pigmented feathers, the transplanted chick leg, for the most part, does not show pigmentation. It is worth mentioning that the transplanted leg of chick origin is often larger than the normal quail leg at the contralateral side. Only PL muscles of those legs have been evaluated whose shape as well as skeletal and muscular pattern corresponded to normal legs. As was described above, in this series, too, histological examinations were performed in order to assure the chicken origin of muscle fibres (Fig. 8). Figure 9 shows the dorsal surface and a fibre bundle of such a chicken PL innervated by appropriate quail motoneurons. Most experiments performed in this series yield PL muscles of chick origin which reveal a quail typical motor endplate pattern.

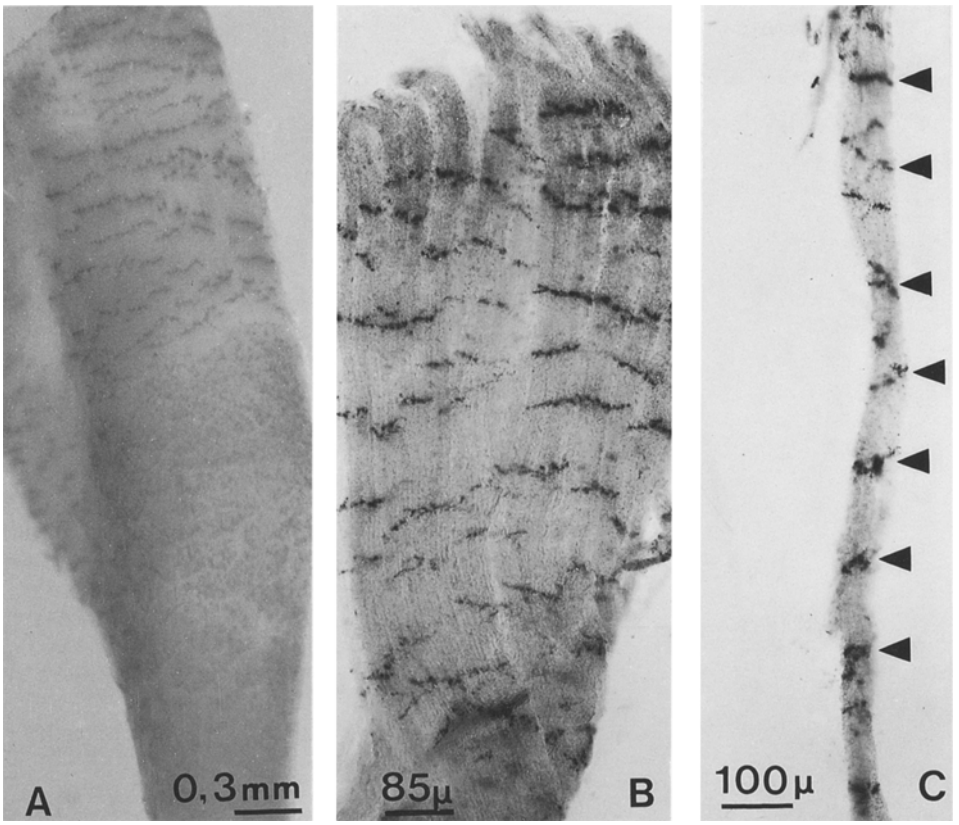
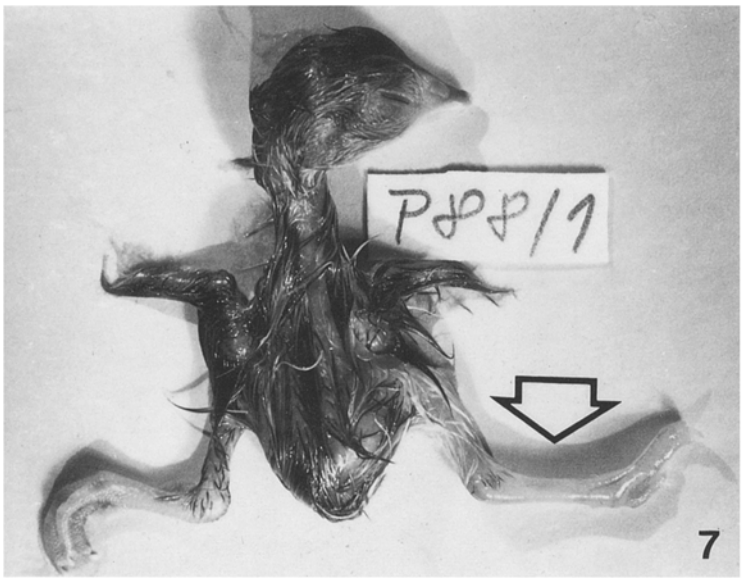


Fig. 6A–C. Chick-like endplate pattern of a quail PL in a quail leg grown in place of the chick leg.
A Dorsal surface of the whole muscle.
B Dorsal part of the PL.
C Muscle fibre bundle. *Arrows*: endplates



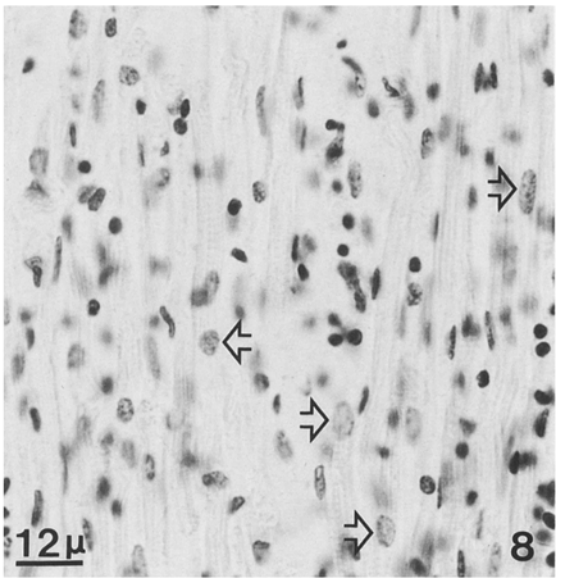


Fig. 7. Quail host after grafting a chick leg (*arrow*) in place of the right quail leg and a subsequent reincubation period of 13 days

Fig. 8. Medial gastrocnemius muscle cut out from a grafted leg. Note the chick nuclei within the myotubes (*arrows*)

According to the findings mentioned above, motoneurons of the host embryo must have dictated the innervation pattern of the PL.

III. PL innervated by inappropriate motoneurons

In the third experimental series leg buds were grafted in place of wing buds using the quail-chick system. Figure 10 shows a quail leg grown instead of the right wing of a chick host. Before fixation it had been noticed that the transplanted leg moved synchronously with the opposite wing. Only those legs have been evaluated, that were found to exhibit a normal shape as well as normal skeletal and muscular pattern. Histological examinations were performed in this series, too (not shown).

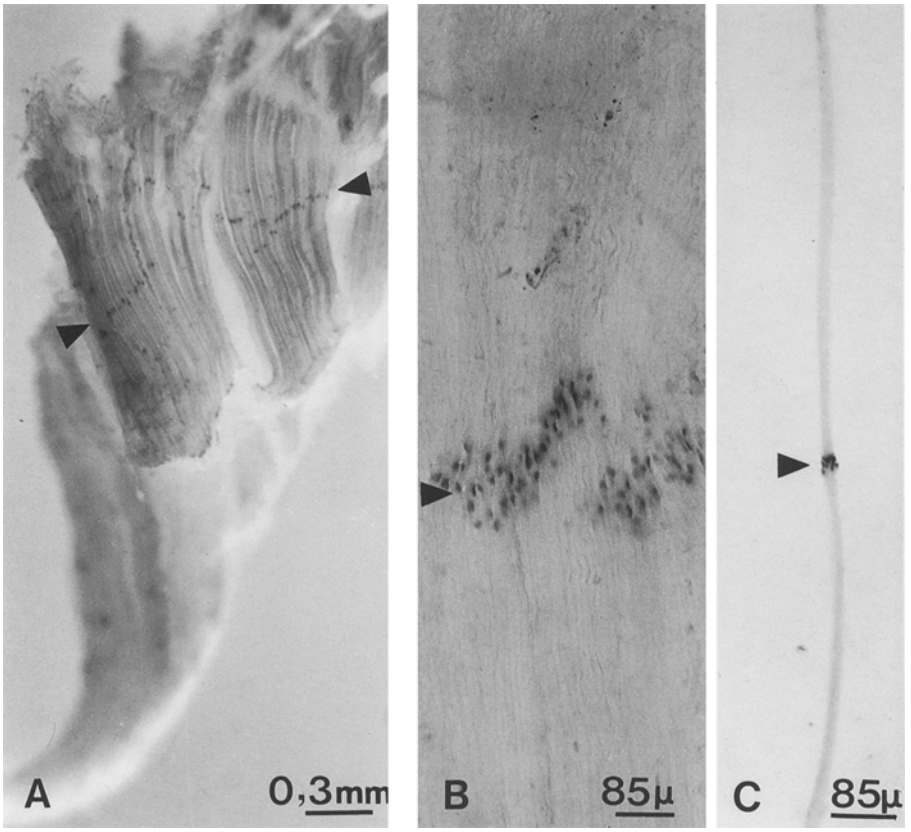


Fig. 9A, B. Quail-like endplate pattern of and chick PL in a chick leg grown in place of the quail leg.
A Dorsal surface of the whole muscle.
B Higher magnification of the dorsal part of the PL.
C Small muscle fibre bundle. *Arrows*: endplates

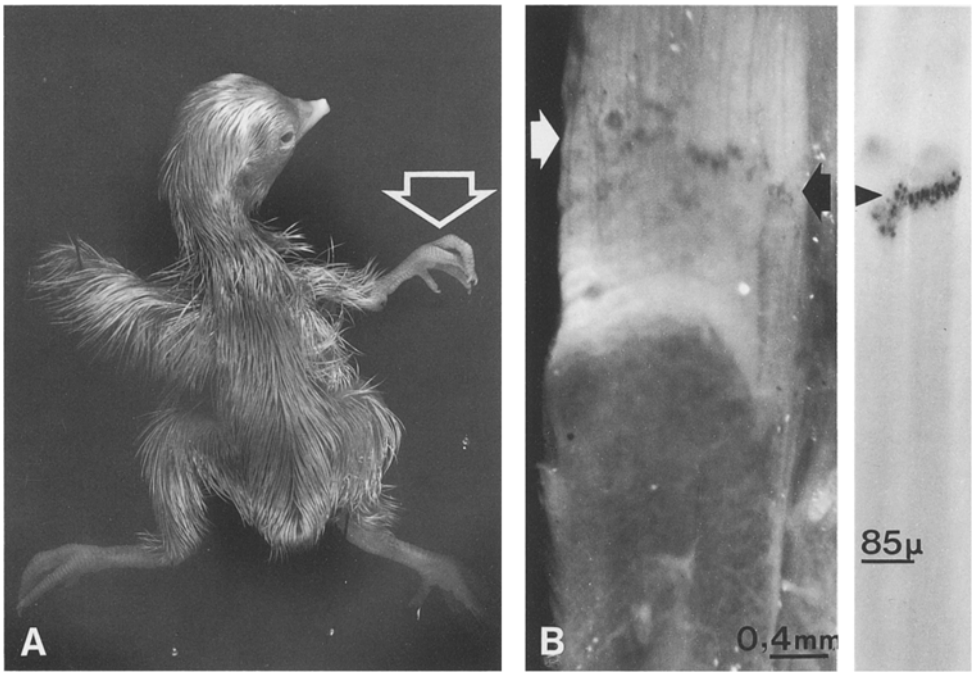
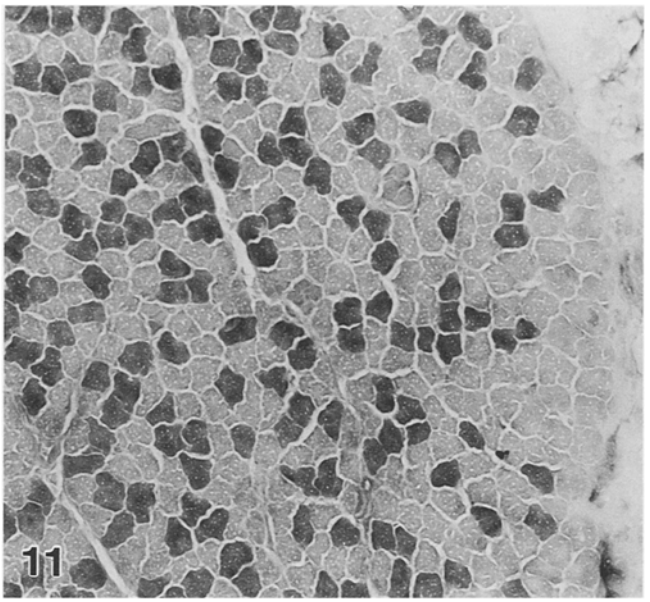


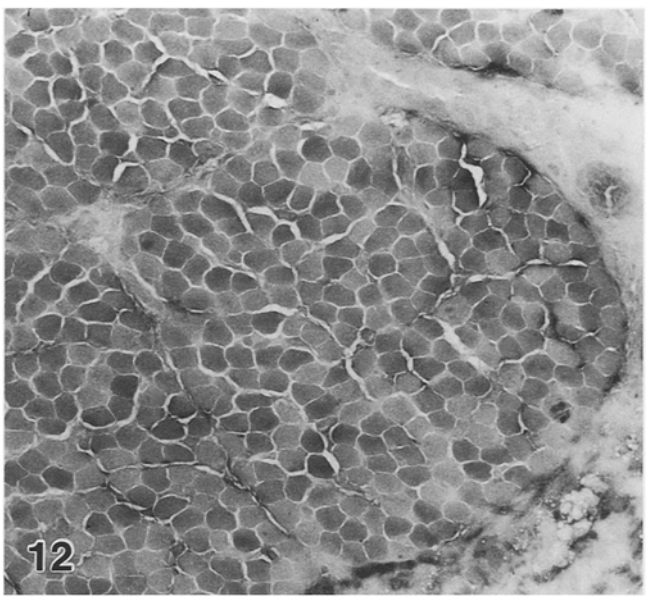
Fig. 10. A Chick host with a quail leg (*arrow*) grown in place of the right wing.
B Dorsal surface of the PL muscle showing a quail-like endplate pattern (*arrows*).
C Bundle of muscle fibres. *Arrow*: endplates

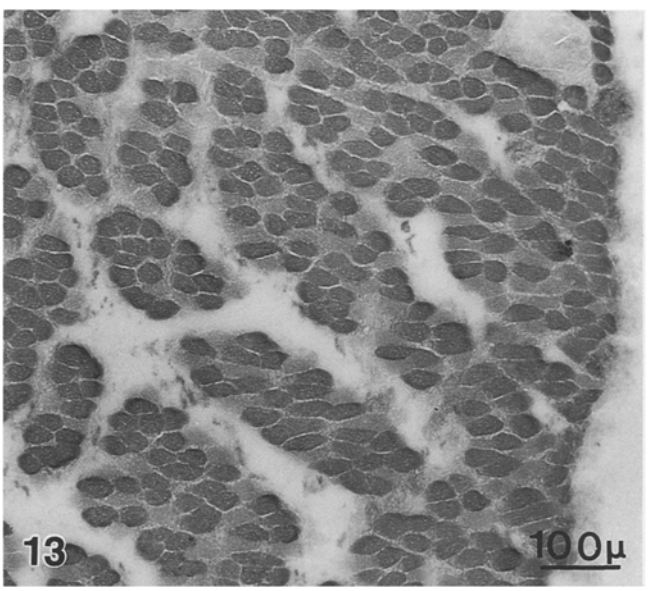
Figure 10 shows the endplate pattern of a PL cut out of a quail leg and innervated by inappropriate motoneurons located in the brachial part of the spinal cord. The most interesting observation that emerged from this series is that an endplate pattern characteristic of the donor PL has developed. That is to say, that inappropriate motoneurons do not have the ability to determine the motor innervation type of the muscle.

IV. Myosin ATPase reaction

According to the classification of Barnard et al. (1982), the PL of the chick is composed of two subclasses of type III-fibres which are multiply innervated (Fig. 11). Fibres which are stained in a medium way belong to the type III A and those which are intensely stained to type III B. The quail PL shows weakly stained (acid-sensitive) type II-fibres







which are focally innervated (Fig. 12). They are especially numerous on the dorsal surface of the muscle. The darkly stained (acid-stable) fibres are multiply innervated and represent III A in III B types. In chimeras in which the PL of quail origin is innervated by appropriate chick motoneurons, muscle fibres can be found which are stained darkly and in a medium way (Fig. 13). These fibres belong to the III A and III B types which are typical of the chick PL. Weakly stained type II-fibres are rare.

V. Localization of PL motoneuron pool

It remains to be checked that in the case of orthotopically grafted legs the PL is actually innervated by appropriate motoneurons. In six chicks to which a quail leg bud had been grafted, the motoneuron pools were made visual using the HRP technique. The PL of grafted quail leg and of the opposite chick leg of E 10 and E 11 hosts were injected with HRP. Labeled motoneurons were examined in transverse serial sections of the spinal cord. In addition, also in E 9 an E 10 quail the motoneuron pool of PL was demonstrated in the same way. In both species the majority of stained motoneurons is found in the lumbosacral segments 4–6 (LS 4–6) (Fig. 14). These motoneurons are grouped in the medial region of the lateral motor column. The position of the PL motoneuron pool of grafted quail legs is in accordance with that found in the contralateral half of the spinal cord with respect to their mediolateral and cranio-caudal extent. It therefore can be stated that the PL of the grafted quail leg innervated by the chick spinal cord received innervation from the appropriate PL motoneuron pool.

Discussion

To get a better understanding of mechanisms involved in the control of the development of muscle specific motoneuron innervation, endplate patterns of the PL in legs that had been previously transplanted between chick and quail embryos are studied. Investigations are based on the observation that PL muscles of chick and quail show different endplate patterns and are made up of distinct muscle fibre types (Grim et al. 1985). The chicken PL consists of multiply innervated slow fibres while the quail PL is mainly composed of focally innervated fast fibres. The experimental design thus far provides the possibility to force appropriate or inappropriate motoneurons of one species to innervate the PL of the other species.

The terms "appropriate" and "native" are used to characterize motoneurons located within the pool from

Fig. 11. Fibre type composition of the dorsal part of the quail PL. Myosin ATPase reaction. Focally innervated type II fibres are weakly stained; multiply innervated type III A and III B fibres are darkly stained

Fig. 12. Fibre type composition of the dorsal part of the chick PL. Myosin ATPase reaction. Muscle is made up of multiply innervated type III A and III B muscle fibres

Fig. 13. Chimeric quail PL innervated by appropriate chick motoneurons. Muscle fibres belong tho the III A and III B types which are typical of the chick PL

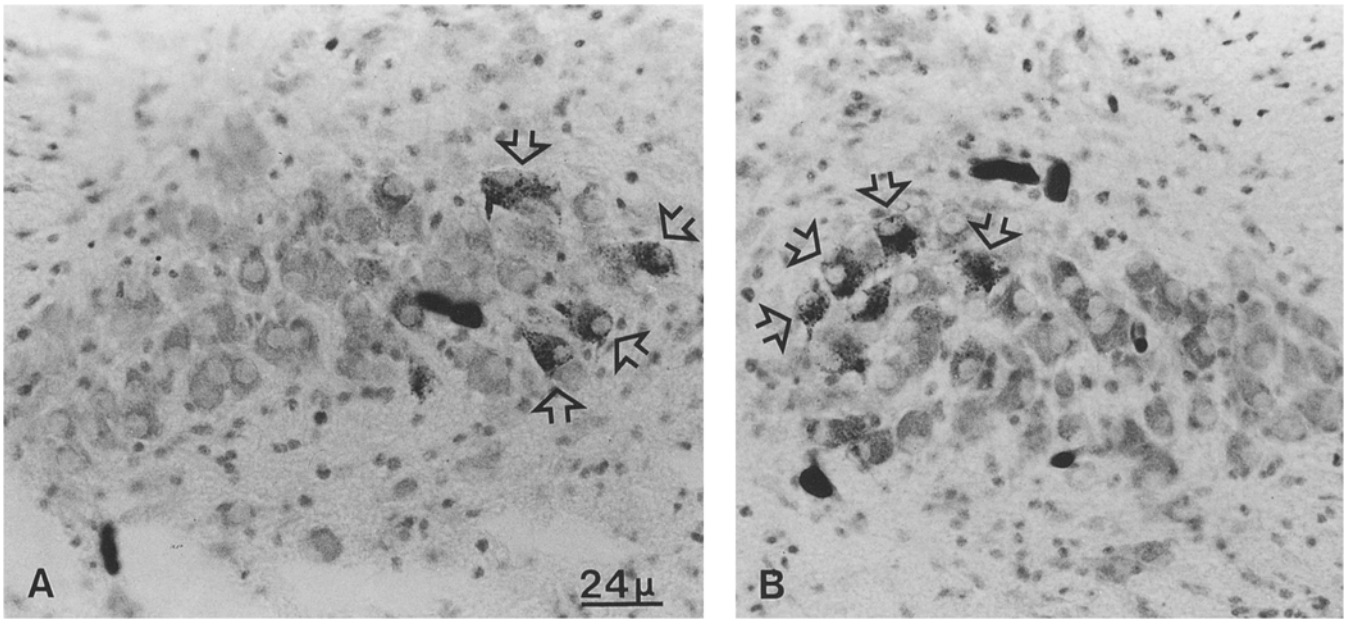


Fig. 14 A, B. Cross section through lateral motor columns (LMC) at the LS level after HPR injecting into PL muscles. *Arrows*: motoneurons of PL pools closely clustered in a discrete medial region of the LMC contain granular reaction product. Cresyl violet staining. **A** Pool of PL motoneurons of the normal left chick leg. **B** Motoneuron pool of the PL within the grafted quail leg of the right side

which the PL is normally innervated regardless of the species. "Foreign" or "inappropriate" motoneurons are those located within spinal cord segments which normally do not contribute to the leg innervation. The effect of the experimentally altered motor innervation is analyzed by staining the endplates. The evaluation of the experimental series yield the following results.

1. PL muscles within quail legs orthotopically transplanted to chick hosts develop an endplate pattern characteristic of the chick.

2. PL muscles within chick legs orthotopically transplanted to quail hosts develop an endplate pattern characteristic of the quail.

3. PL muscles within legs heterotopically transplanted develop an endplate pattern characteristic of the donor embryo.

4. PL muscles within transplanted legs are always found to be mainly made up of donor myocytes.

5. The myosin ATPase reaction shows that appropriate motoneurons are able to alter the fibre type.

6. The location of the motoneuron pool innervating the PL within grafted quail legs corresponds to the location of the motoneuron pool innervating the normal chick PL.

Thus, our results show that appropriate motoneurons in avian chimeras are able to dictate a muscle innervation type that normally does not occur. Unlike this dictatorial influence of appropriate motoneurons inappropriate motoneurons do not determine the endplate pattern of the muscle. In this case the endplate pattern corresponds to that found in the normal leg. From these results it can be deduced that at least two different influences controlling endplate pattern formation can become effective, a central and a peripheral one.

Our findings concerning the dictatorial influence of motoneurons on development of focally or multiply innervated muscle fibres are in line with the cross-innervation experiments performed by Hnik et al. (1967), Jirmanová et al. (1971)) and Jirmanová and Zelená (1973) who transplanted the nerves between the multiply innervated ALD and the focally innervated PLD using adult and especially young chicken. Under these experimental conditions, a focal innervation develops in the ALD and a multiple one in the PLD. Recently, Vogel and Landmann (1987) got some evidence that under exceptional circumstances after a motoneuron-muscle fibre type mismatch, embryonic motoneurons can alter fibre type expression.

Various observations exist that point to an involvement of muscle primordia itselves in determining the fibre types and endplate pattern during embryonic development. Christ et al. (1983) showed that after replacement of the brachial neural tube by leg level neural tube in the avian embryo, the UMD of the wing does not change its normal motor innervation pattern. This muscle is characterized by a multiply innervation dorsal and a focally innervated ventral part (Grim et al. 1983). Similar results were obtained after grafting the wing bud in place of the leg bud (Jacob et al. 1983; Laing and Lamb 1983; Grim et al. 1986). In all cases the inappropriately innervated UMD exhibits a normal, that is to say an unchanged endplate pattern. Moreover, despite the foreign innervation the subsequent development of the distribution of fast and slow muscle fibres, as judged by ATPase staining, was normal in all muscles examined (Laing and Lamb 1983). Butler et al. (1986) maintained that "naive nerves lacked the memory of a previous partner" and thus, cannot alter fibre types if they are forced to innervate inappropriate muscles. Studying aneurogenic limbs, Christ et al. (1979) found a normal muscle pattern. Examining such muscles by means of myosin ATPase histochemistry, Butler et al. (1982) observed that in those muscles, lacking any peripheral neuronal influences, the initial differentiation of distinct fibre types does occur.

If we favour the model of intrinsic fibre type differentiation, the question arises how the myotubes become determined. Studies concerning limb muscle development have

shown clearly that the myoblasts originate from the somites while the total of the muscular connective tissue derives from the somatopleure (Christ et al. 1974, 1977; Chevallier et al. 1976, 1977). The somite-derived myoblasts were found to be "naive" with respect to their destination within the limb (Chevallier et al. 1977; Christ et al. 1978; Lance-Jones 1988a, b). After heterotopical substitution of brachial somites in a chick host by non-brachial quail somites, the chimeras show a normally developed chick-specific muscle pattern and the muscles are made up of quail myocytes (Jacob et al. 1983). Development of individual muscle form is brought about by influences mediated by the somatopleure-derived connective tissue of the limb (Jacob and Christ 1980). Such chimeric wings also show a normal endplate pattern. It is therefore suggested that the developing myotubes become informed of their spatial arrangement and of their fibre types by local cues mediated by cell-cell or cell-matrix interactions (Christ et al. 1986). This has been recently supported by Butler et al. (1988).

Looking at the results mentioned above, it can be suggested that the motoneurons determine the muscle fibre types. On the other hand, the significance of peripheral influences for the muscle specific endplate pattern formation is experimentally well documented. The results on the PL obtained with quail-chick chimeras offer a possibility to get out of this controversial issue. We set out a model proposing a hierarchy of factors controlling motoneuron innervation.

If appropriate motoneurons are allowed to project to the PL, that species-specific endplate pattern is expressed which is inherent in the motoneuron pool. In this case, the centrally situated information wins through against the peripherally located programme. During normal development of motoneuron innervation the centrally and peripherally located programmes are identical, what might be the reason for the generation of the high neuromuscular specificty.

If inappropriate motoneurons are forced to project to the PL, the species-specific pattern is realized that is inherent in the muscle primordium itself. In the latter case, the peripherally located information wins through against the central one, on condition that muscle fibres are in the position to choose appropriate partners. In the vast majority of experiments such connections seem to be set up. Under these circumstances which do not exist during normal development axons may compete with each other (for review: Bennett 1983). According to the statement made by Vrbová et al. (1978) there "is a clear preference of slow nerve fibres for slow muscle fibres and fast nerve fibres for fast muscle fibres". Muscle cells differentiated within the limbs are supposed to possess identities, detectable by differences in myosin ATPase content, before they are innervated (Bennett 1983). If under experimental conditions, the number of corresponding motoneurons is not sufficient, it may happen that muscle fibres are forced to accept a wrong partner. This might explain the motoneuron-muscle fibre type mismatch leading to an alteration of fibre type expression in some muscles as was observed by Vogel and Landmesser (1987) and several other authors who performed cross-innervation experiments in young chickens and points to a plasticity of muscle fibres to some extent under the influence of a separate axon information.

According to Landmesser (1978) and Hollyday (1980) motoneurons exhibit target selectivity. If inappropriate motoneurons mismatch a foreign muscle their first choice is a muscle fibre of the corresponding type. In case they do not find the right partner second choice innervations will develop. This might be interpreted as an expression of a hierarchy of neuronal specifities (Hollyday et al. 1977).

According to the hierarchy concept given in this paper, firstly muscles innervated by appropriate motoneurons express an endplate pattern which is established within the pool. We therefore cannot support the suggestion of Butler et al. (1986) that motoneurons projecting to muscle primordia are judged to be naive with respect to slow and fast characteristics. If, secondly, the centrally located programme cannot be realized the peripheral programme is found to dominate. That may best explain the stability of the endplate pattern seen in the UMD of heterotopically grown wings (Jacob et al. 1983; Laing and Lamb 1983; Grim et al. 1986). Thus, realization of neuromuscular synapse formation is suggested to be molecularly based on recognition processes between axons of different motoneurons and different muscle cells. One might assume that motoneurons and myogenic cells are uniquely labeled in accord with their axial level of origin early in development (Lance-Jones 1988a, b). As was mentioned above muscle precursor cells of the somites were found to be blank with respect to their level. However, the possibility exists that the somatopleure which subsequently form the connective tissue and the substrates of the highways are marked by level-specific signals.

Reviews in which HRP techniques are discussed (Hollyday 1980; Landmesser 1980) show that motoneurons laterally situated within the lateral motor column (LMC) project to muscles arising from the dorsal premuscular mass while motoneurons medially situated within the LMC, project to muscles arising from the ventral premuscular mass. The PL as well as the medial part of the gastrocnemius muscle of the chick were found to come from a common muscle primordium which originates from the ventral premuscular mass (Pautou et al. 1982). This must also be true for the quail. Therefore, the species-specific origin of the PL in quail-chick chimeras can be judged by histological examinations of pieces cut out from the medial part of the gastrocnemius muscle.

In accordance with the developmental origin of the PL in both species the motoneuron pool of the PL was found in the medial part of the LMC. Tanaka and Landmesser (1986) showed that in chick-quail chimeras motoneuron pools of one species selectively innervate homologous muscles in the limb of the opposite species with considerable precision. After transplantations of the quail leg bud in place of chick leg bud the motoneurons innervating the quail PL were situated in a position which corresponds to the pool of the normal chick PL. Therefore, it is likely that the PL of the quail became innervated by appropriate motoneurons.

Looking at the results obtained with the PL in quail-chick chimeras we feel that the concept of a hierarchical control of motoneuron innervation might help to reconcile the different experimental results and controversial theories dealing with this subject.

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