J. Anat. (1997) **191**, pp. 507–515, with 2 figures Printed in the United Kingdom

Review

Role of the extracellular matrix in neural crest cell migration

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(*Accepted 8 September 1997*)

ABSTRACT

Development of the neural crest involves a remarkable feat of coordinated cell migration in which cells detach from the neural tube, take varying routes of migration through the embryonic tissues and then differentiate at the end of their journey to participate in the formation of a number of organ systems. In general, neural crest cells appear to migrate without the guidance of long-range physical or chemical cues, but rather they respond to heterogeneity in the extracellular matrix that forms their migration substrate. Molecules such as fibronectin and laminin act as permissive substrate components, encouraging neural crest cell attachment and spreading, whereas chondroitin sulphate proteoglycans are nonpermissive for migration. A balance between permissive and nonpermissive substrate components seems to be necessary to ensure successful migration, as indicated by a number of studies in mouse mutant systems where nonpermissive molecules are over-expressed, leading to inhibition of neural crest migration. The neural crest expresses cell surface receptors that permit interaction with the extracellular matrix and may also modify the matrix by secretion of proteases. Thus the principles that govern the complex migration of neural crest cells are beginning to emerge.

Key words: Neural crest; extracellular matrix; cell migration.

INTRODUCTION

Neural crest cells arise in the dorsal midline of the embryonic neural tube, from a stem cell population that also produces neurons and glia (Stemple & Anderson, 1992). The neural crest delaminates from the neural tube by a process of epithelial to mesenchymal transformation (Duband et al. 1995) and exits into a cell-free space beneath the surface ectoderm. The crest cells then migrate peripherally, in some cases traversing considerable distances within the embryo, before contributing to a variety of tissue and organ systems (Hörstadius, 1950; Weston, 1970; Le Douarin, 1982).

Neural crest cells follow specific pathways to their destinations within the embryo (Erickson & Weston, 1983). For instance, in the trunk region, the early migrating neural crest passes between the dermomyotome and the neural tube, either taking a route close to the neural tube on the ventromedial pathway, or close to the dermomyotome on the ventrolateral pathway (Serbedzija et al. 1990) (Fig. 1). The migration of this ventral neural crest is further restricted in the rostrocaudal axis, with cells migrating only through the rostral part of each sclerotome (Rickmann et al. 1985). Later migrating cells follow a dorsolateral pathway, outside the developing dermomyotome, and subsequently give rise to melanocytes. In this paper, we review the current state of knowledge concerning the developmental mechanisms that determine the characteristic pathways taken by migrating neural crest cells.

NEURAL CREST CELL MIGRATION

Neural crest cells migrate actively, without using long-range guidance cues

The control of neural crest cell migration has long been a subject of speculation and experimentation. Although the neural crest could be passively translocated by morphogenetic movements in associated tissues (Noden, 1984), such a model does not easily

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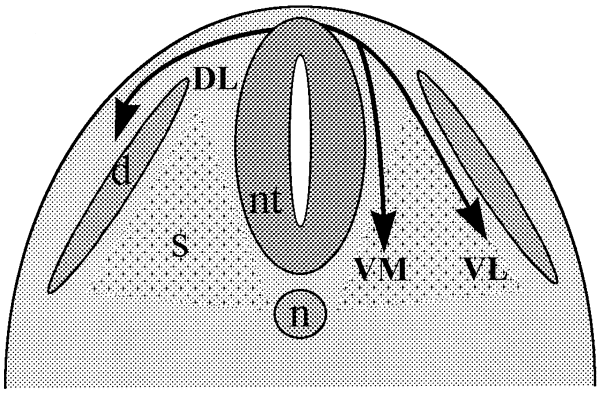

Fig. 1. Diagrammatic representation of the principal pathways of neural crest migration as viewed in the transverse plane of the avian and mammalian embryonic trunk region. The first wave of neural crest emerges from the dorsal neural tube (nt) and migrates ventrally between the dermomyotome (d) and the neural tube, following either a ventromedial pathway (VM) close to the neural tube or a ventrolateral pathway (VL) along the dermomyotome basement membrane. Later migrating cells pass lateral to the dermomyotome on the dorsolateral pathway (DL), giving rise predominantly to melanocytes. The mesenchyme immediately surrounding the notochord (n) and the caudal portion of each sclerotome (s) do not support neural crest migration, owing to the high concentration in these tissues of nonpermissive chondroitin sulphate proteoglycans.

explain how neural crest cells approach their targets in the periphery, when major morphogenetic movements have ceased. Moreover, the active migration of neural crest cells in vitro has been extensively demonstrated (Davis & Trinkaus, 1981; Greenberg et al. 1981; Erickson & Olivier, 1983; Ito & Takeuchi, 1984). The consensus view is, therefore, that neural crest cells move actively through their environment during the majority of their migration schedule.

Various possible types of physical guidance cue have been investigated including electrical fields and steric influences. Early studies suggested that electrical fields exist around the neural tube at the time of neural crest migration, and that neural crest cells will migrate towards the cathode if an electric field is imposed in vitro (Erickson & Olivier, 1983). However, there has been no demonstration of a role for electrical fields in guiding neural crest cells in the embryo. Barrier tissues for neural crest, such as the dermomyotome, might sterically restrict cell migration (Newgreen, 1989), an idea that is supported by the accumulation of cells along tissue ridges and grooves, rather than adjacent to, or crossing them (Dunn, 1982). However, Newgreen (1989) suggested that spatial restriction of cell movement is unlikely to have a major impact on neural crest cell migration in vivo since the extracellular spaces in neural crest pathways are easily large enough to accommodate the migrating crest cells.

The predominant idea that emerges is one of active neural crest migration, without the use of global guidance cues, but probably with contact inhibition of cell movement (Abercrombie, 1965) ensuring dispersion of the cells following their delamination from the neural tube. Contact inhibition implies that cells will tend to migrate from areas of high to low cell density and, although the evidence for contact inhibition of neural crest cells in vitro is controversial (Davis & Trinkaus, 1981; Newgreen, 1989), this model closely reflects the migration patterns of neural crest cells as observed in vivo (Tosney, 1978; Serbedzija et al. 1990). Moreover, when the neural crest is ablated from a particular region, cells from adjoining axial levels will fill the gap, supporting the idea of a release of cells from contact inhibition in this case (Couly et al. 1996).

Neural crest cells are guided by their migration substrate, not by diffusible chemotactic factors

The directionality of neural crest migration could be governed by diffusible factors that attract or repel the crest cells, or by heterogeneity within the extracellular substrate on which they migrate. Gradients of chemoattractant would need to exist over large distances in order to explain the frequently long migration pathways of neural crest cells. To date, the only example of such a gradient concerns Stem Cell Factor which appears to attract melanocyte precursors over relatively short distances into the dorsolateral pathway in mouse embryos (Wehrle-Haller & Weston, 1995). There are currently no known examples of diffusible molecules, synthesised in the periphery, that form a gradient capable of guiding neural crest cell migration over large distances. Moreover, avian neural crest cells grafted into the ventral pathway have been shown to migrate in both dorsal and ventral directions, suggesting that chemotactic gradients do not exist, at least in the early stages of migration (Erickson et al. 1980).

Haptotaxis, the process by which cells move along an adhesive gradient, is an alternative mode of guidance of neural crest migration. Although such gradients have not yet been demonstrated in vivo, there is much experimental support for the existence of regional variations in adhesive properties of the extracellular environment through which neural crest cells migrate. The basic idea is that the extracellular matrix comprises molecules that provide permissive or nonpermissive substrates for neural crest migration. These molecules are proposed to line migratory pathways in varying combinations and, depending on

the ratio of the permissive to nonpermissive components, they either repel or permit migration along specific routes. Evidence for the operation of such a mechanism has come from experiments in which latex beads, injected into the pathways of neural crest migration in chick embryos, were found to move ventrally at varying rates depending on the molecule with which they were coated (Bronner-Fraser, 1982, 1984). Beads coated with fibronectin or laminin moved only very short distances from the injection site, whereas those coated with molecules which do not readily support cell adhesion migrated large distances. These findings suggest a mechanism whereby cell adhesion to the substrate is of major importance in determining the extent and rate of forward migration. The mechanisms by which the extracellular environment governs both the timing and direction of neural crest migration is examined in detail in the remainder of this review.

Regional differences in the extracellular environment guide neural crest cell migration

There is growing evidence that various embryonic tissues act as 'barriers' to neural crest cell migration, thereby serving to direct the passage of the neural crest into specific migration pathways. These barrier tissues are inhibitory not only to cell migration but also to the outgrowth of axons, suggesting parallel mechanisms in these 2 systems. Barrier tissues include the caudal part of the sclerotome, the dermomyotome and the perinotochordal mesenchyme (Newgreen et al. 1986; Lofberg et al. 1989; Pettway et al. 1996). The streaming of neural crest through the rostral part of each somite, with avoidance of the caudal portion, is a characteristic aspect of crest cell migration and experiments in which somites were rotated about their rostrocaudal axis demonstrate that this heterogeneity is intrinsic to the somite (Stern et al. 1991). Furthermore, migration is inhibited when various barrier tissues are transplanted into the pathways of avian neural crest. For instance, transplants of segmental plate mesoderm are inhibitory to neural crest migration whereas this inhibitory effect is lost once the paraxial mesoderm differentiates into sclerotome, suggesting a stage-dependent variation in the inhibitory effect of paraxial mesoderm (Bronner-Fraser & Stern, 1991). Similarly, the notochord repels neural crest migration as demonstrated by the observation that neural crest cells avoid a transplanted notochord fragment in the chick (Pettway et al. 1990). The question then arises: what are the molecules that permit or inhibit neural crest migration, which exhibit region-specific distribution in the embryo ensuring directed pathways of neural crest migration?

Permissive extracellular matrix molecules

Fibronectin, laminin and some collagen isoforms are all expressed along pathways of neural crest cell migration and are suggested to be permissive for neural crest cell migration (Newgreen & Thiery, 1980; Krotoski et al. 1986; Sternberg & Kimber, 1986; Tuckett & Morriss-Kay, 1986; Perris, 1997). This idea is supported by experimental studies in which short peptides corresponding to the cell-binding domain of fibronectin were shown to prevent the migration of neural crest cells in vivo after injection into chick embryos (Boucaut et al. 1984). The peptides probably saturate cell surface receptors for fibronectin on the neural crest cell surface, thereby preventing the interaction of the cells with the permissive fibronectin molecule. Experiments in vitro have complemented the in vivo studies by showing that fibronectin, collagens and laminin all support the attachment, spreading and migration of isolated neural crest cells (Greenberg et al. 1981; Perris et al. 1989, 1996*a*). Collagen types I, III and IV are all expressed in the right time and place to be substrates for neural crest migration (Newgreen & Erickson, 1986*a*; Perris et al. 1991, 1993*a*) but, in vitro, collagens do not seem as good a substrate for neural crest migration as fibronectin. Laminin is an excellent substrate for neural crest cells in vitro and the association of migrating neural crest cells with basement membranes supports a physiological role for laminin in directing neural crest cell migration (Duband & Thiery, 1987; Erickson, 1988). Laminin molecules are glycoproteins comprised of three component chains (α, β and γ). Laminins were recently renamed according to a new nomenclature, such that for example laminin-1 is comprised of α1, β1 and γ1 chains, whereas laminin-4 is made up of α2, β2 and γ1 chains (Burgeson et al. 1994). Each laminin has a separate distribution although most are associated with the epithelial basement membrane. Interestingly, different isoforms may be expressed pre and postnatally (Cooper & MacQueen, 1983; Leivo & Engvall, 1988), and there may be a switch between 2 isoforms as development proceeds (Miner & Sanes, 1994). Most experiments on the effects of laminin on neural crest cell migration in vitro have not distinguished between variants, although in most cases it is likely to have been the prototype laminin, laminin-1.

Neural crest cells express cell surface molecules that are capable of interacting with extracellular matrix

components, in particular receptors of the integrin family which variously bind fibronectin, laminin and other extracellular matrix molecules (Lallier & Bronner-Fraser, 1991; Lallier et al. 1992). Integrins are expressed on neural crest cells and appear to be necessary for the neural crest to attach to the substratum in vitro and to migrate in vivo (Lallier et al. 1994; Stepp et al. 1994). Studies using antisense oligonucleotides specific for various integrins have implicated at least 5 α1 integrins and 3 β1 integrins in the interaction of neural crest cells with fibronectin and laminin in vitro (Lallier & Bronner-Fraser, 1993; Delannet et al. 1994; Kil et al. 1996). Moreover, injection of antisense oligonucleotides in vivo detrimentally affects neural crest migration in the chick embryo (Kil et al. 1996). In conclusion, it seems likely that integrins are an important component of the machinery by which neural crest cells interact with permissive substrate molecules in vivo, although the precise nature of the integrin species involved remains to be determined.

Nonpermissive extracellular matrix molecules

There have been extensive studies aimed at determining the active molecular components of barrier tissues for neural crest cell migration. Although tenascin was originally suggested as a candidate for the inhibitory activity demonstrated in the caudal part of the somite (Tan et al. 1987), subsequent studies demonstrated that it is not the active molecule (Stern et al. 1989). Carbohydrates that bind to the lectin peanut agglutinin and chondroitin-6-sulphate glycosaminoglycan chains are also abundant in barrier tissues for neural crest cell migration and axonal outgrowth (Oakley & Tosney, 1991; Oakley et al. 1994; Krull et al. 1995). Expression of these glycoconjugates correlates with delayed entry of the neural crest into the dorsolateral pathway and experimental deletion of the dermomyotome, with which these glycoconjugates are associated, results in enhanced neural crest migration along this pathway (Oakley et al. 1994).

Recently, these peanut agglutinin-binding, chondroitin sulphate-bearing molecules have been identified as the extracellular matrix proteoglycans aggrecan and versican (Tan et al. 1987; Ranscht & Bronner-Fraser, 1991; Landolt et al. 1995; Pettway et al. 1996). Aggrecan exists in at least 2 splice forms that are differentially expressed both spatially and temporally. One variant is cartilage-specific, whereas the other is associated with the notochord and is thought to be responsible for the repulsive effects of the perinotochordal mesenchyme on both neural crest cell migration and axonal outgrowth. Pettway et al. (1996) showed recently that the notochord-associated variant itself exists in 2 isoforms which carry different keratan-sulphate side chains and carbohydrate moieties, and which have temporally regulated expression patterns. The first isoform co-distributes with the HNK-1 antigen and is strongly inhibitory to neural crest cell migration whereas the second isoform is expressed later, no longer bears the HNK-1 epitope and does not inhibit neural crest cell migration. It has also been shown that aggrecan is strongly inhibitory to avian neural crest cell migration in vitro (Perris et al. 1996*b*). This inhibition of neural crest migration appears to be independent of substrate binding and is likely to be mediated through the glycosaminoglycan side chains.

Versican is another member of the family of chondroitin sulphate proteoglycans. It is expressed in the chick in barrier tissues to neural crest cell migration, such as the caudal sclerotome and the perinotochordal mesenchyme (Landolt et al. 1995). Moreover, as with aggrecan, once neural crest cell migration is completed, expression of versican becomes associated with prechondrogenic areas. Recent in situ hybridisation studies demonstrate that the versican gene is expressed in a similar distribution in the mouse as in the chick (D. J. Henderson & A. J. Copp, unpublished results). Versican is nonpermissive for neural crest attachment in vitro (Perris et al. 1996*b*) but is less inhibitory than aggrecan, this difference being attributed to the higher content of keratan sulphate side-chains in aggrecan, whereas versican has predominantly chondroitin sulphate chains.

Mutants reveal a role for the extracellular matrix in guiding neural crest cell migration

A number of mouse mutants have abnormalities of neural crest migration as part of their phenotype. These mutants have highlighted the importance of the extracellular environment in controlling neural crest cell migration. Although there are no known mutations in the versican gene, analysis of the *Pax3* mouse mutant, *splotch* (Sp^{sH}) is proving useful in elucidating the role of versican in development. *Splotch* homozygotes have a variety of developmental defects, including spina bifida and exencephaly, absence of limb musculature and deficiency of neural crest cell derivatives. Homozygotes present with reduced or

absent dorsal root ganglia, diminished Schwann cell numbers, pigmentation defects, and failure of neural crest colonisation of the outflow tract of the heart, resulting in persistent truncus arteriosus (Chalepakis et al. 1993). However, *Pax3* mutations affect only a subset of neural crest cells as the cranial skeleton is largely normal. Trasler & Morriss-Kay (1991) described elevated levels of heparan sulphate and chondroitin sulphate proteoglycans in *splotch* embryos and, in recent gene expression studies using whole mount in situ hybridisation, we have identified versican as the proteoglycan primarily affected in *splotch* embryos (Henderson et al. 1997). Versican is over-expressed in Sp^{2H} homozygotes in pathways of neural crest cell migration, and is also present in ectopic sites. Specifically, versican expression is seen at elevated levels in the dorsal mesoderm underlying the surface ectoderm, where neural crest cells first emerge from the neural tube, and in the regions surrounding the dorsal root ganglia and the dermomyotome. The overexpression of versican suggests a mechanism in which the neural crest cells in Sp^{2H} homozygotes are prevented from migrating by an over-abundance of nonpermissive molecules in their migratory route.

Proteoglycans also accumulate abnormally in other mouse mutants with neural crest cell migration defects. Mice homozygous for the *patch* mutation (*Ph*) resemble *splotch* in having pigmentation abnormalities and persistent truncus arteriosus, although neural tube closure appears to occur relatively normally (Gruneberg & Truslove, 1960). *Patch* mice have a deletion of the gene encoding platelet-derived growth factor receptor α (PDGFα), a defect that affects a subset of nonneuronal neural crest-derived cells, mainly in the cranial region (Morrison-Graham et al. 1992). This mutant has also been shown to have abnormal levels of extracellular matrix components in the pathways of neural crest cell migration. It appears that neural crest cells do begin to migrate away from the neural tube in the *Ph* mutant, but electron microscopy has revealed increased accumulation of glycosaminoglycan-containing granules in the mutant embryos (Morrison-Graham et al. 1992). This increase was evident before the onset of neural crest cell migration, suggesting it may be a cause, and not a secondary effect, of abnormal development of crest-derived structures.

The *white spotting* (*W*) and *steel* (*Sl*) mutants also exhibit defective migration of neural crest cells, specifically melanocyte precursors, resulting in abnormal pigmentation (Jackson, 1994). *W* mice have mutations in *c-kit*, a cell-surface receptor with intrinsic tyrosine kinase activity, whereas *Sl* mice have mutations in the ligand for *c-kit*, named Steel Factor or Stem Cell Factor (SCF) (Nocka et al. 1990; Reith et al. 1990; Zsebo et al. 1990). SCF is a transmembrane protein which also exists in a soluble form. The ligand-receptor interaction results in protein phosphorylation of cytoplasmic proteins which contain SH2 domains. Mutations in either gene result in abnormal signal transduction through the pathway, and are responsible for the phenotypes of the *W* and *Sl* mutants (Morrison-Graham & Takahashi, 1993). SCF is transiently expressed in the dorsolateral migration pathway, between the dermomyotome and the surface ectoderm, before the initial immigration of melanocyte precursors (Wehrle-Haller & Weston, 1995). In the absence of SCF, although the melanocytes precursors emigrate from the neural tube, they fail to enter the dorsolateral pathway, suggesting that the soluble form of SCF is a chemoattractant for melanocyte precursors, whereas the membrane bound form may act as a survival factor (Duttlinger et al. 1993, 1995; Wehrle-Haller & Weston, 1995; Wehrle-Haller et al. 1996).

The *lethal spotting* (*ls*/*ls*) and *piebald lethal* (s^l/s^l) mouse mutants have abnormalities of pigmentation and anomalies of the enteric nervous system resulting in absent or diminished innervation of the gut. These mice have mutations in the endothelin-3 and endothelin-B receptor genes, respectively (Baynash et al. 1994; Hosoda et al. 1994), and resemble humans with Hirschsprung's disease, many of whom have mutations in the endothelin-B receptor (Puffenberger et al. 1994). These mutations cause over-abundance of extracellular matrix components in the enteric basement membrane, including laminins, collagen type IV, perlecan and other proteoglycans (Payette et al. 1988; Tennyson et al. 1990). Mice mutant for the *c-ret* oncogene, which have a very similar type of aganglionic megacolon, do not exhibit this abnormal accumulation of extracellular matrix, indicating that the matrix defect is unlikely to be a secondary affect of aganglionosis (Rothman et al. 1996). These findings suggest that whereas the *c-ret* mutation may involve a defect intrinsic to the neural crest cells, the *ls* and s^l mutations result in an abnormality of the extracellular environment through which the neural crest cells migrate.

In contrast to the mouse mutants described above, the *white* axolotl mutant has a reduction in the accumulation of extracellular matrix proteins in the pathways of neural crest cell migration. Pigment cells of the *white* mutant embryo migrate only short distances from their site of origin in the dorsal neural

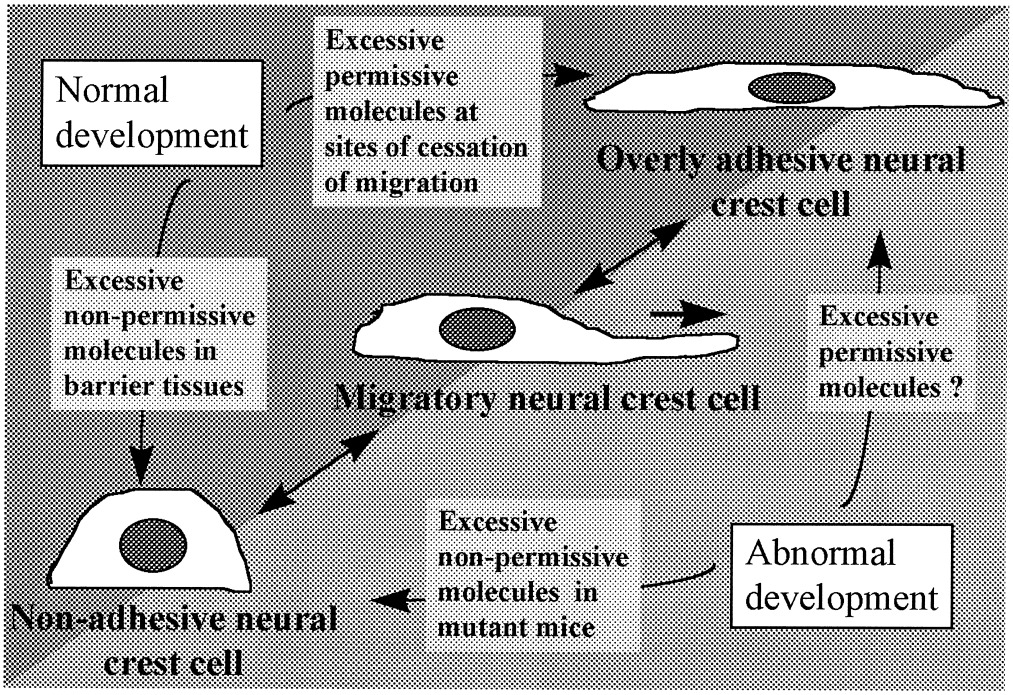

Fig. 2. Cartoon depicting the critical nature of the balance between permissive and nonpermissive molecules in the extracellular matrix for ensuring successful migration of the neural crest. Excessive quantities of nonpermissive molecules such as chondroitin sulphate proteoglycans are incompatible with neural crest migration during normal development (i.e. in 'barrier' tissues), thereby serving to direct migration into specific pathways. In pathological situations overexpression of nonpermissive molecules may lead to overall failure of neural crest cell migration, as in the *splotch* mutant. Permissive molecules may be expressed at elevated levels at the sites of cessation of migration during normal development and, in principle, could lead to premature arrest of neural crest migration in situations of pathological overexpression, although currently there are no clear examples of this type of defect.

tube, although migration on the ventromedial pathway between the neural tube and the somites appears to be completely normal. This highlights a particular region of subepidermal extracellular matrix as the cause of the neural crest migration failure in this mutant. When extracellular matrix from the subepidermal region of wild type and *white* mutant embryos was adsorbed onto membrane microcarriers and transplanted into host embryos in various combinations, grafts from mutant to wild-type embryos inhibited neural crest cell migration (Lofberg et al. 1989). This effect was also shown to be stage-dependent indicating that the defect in the extracellular matrix in the subepidermal region of the *white* mutant is transient, perhaps suggesting that maturation of the matrix in this region is delayed (Lofberg et al. 1989). Further studies have revealed abnormal levels of versican-like proteoglycans in the subepidermal extracellular matrix of the *white* mutant. Although the primary mutation does not appear genetically linked to versican, it could involve an enzyme involved in posttranslational modification of the extracellular matrix (Perris, 1997). Thus analysis of mutant systems has highlighted the consequences of abnormal matrix deposition on the migration of neural crest cells in vivo, and has suggested that both over and underabundance of specific matrix components can be detrimental to neural crest migration.

SYNTHESIS AND CONCLUSION

The guidance of neural crest migration is a complex control system, in which the neural crest interacts with a variety of extracellular matrix components. In this review, we have concentrated on the effect of varying matrix composition on migration of the neural crest, but perhaps equally important is the ability of the neural crest to modify the matrix through which it migrates. Neural crest cells produce plasminogen activator (Valinsky & Le Douarin, 1985; Erickson & Isseroff, 1989; Menoud et al. 1989), a serine protease that is probably involved in matrix remodelling, but this action of the neural crest is as yet poorly defined. To ensure successful neural crest migration, each matrix molecule appears to be required in specific quantities, at particular locations, according to a tightly regulated temporal programme of development. Moreover, expression of the various molecules must be precisely coordinated with each other. It seems most likely that a balance between permissive

and nonpermissive molecules is the crucial factor (Fig. 2). Permissive molecules allow cell attachment and spreading, whereas nonpermissive molecules are necessary to ensure that attachment to the substrate is not so tight as to preclude forward migration. The mutant studies confirm that excessive quantities of nonpermissive molecules can inhibit migration, and it seems likely that over-abundance of permissive molecules may also compromise migration, as a result of over-adhesion. Indeed, in vitro studies in which the interaction between neural crest cells and the matrix were experimentally manipulated show that excessive adhesion results in suppression of neural crest cell movement, whereas lower levels of adhesion result in increased rates of migration (Perris et al. 1993*b*). Lack of a molecule may be of less functional importance than over-expression, as suggested by the apparently normal neural crest derivatives that are formed in mice homozygous for a null mutation in the fibronectin gene (George et al. 1993). In this case, other permissive matrix components may substitute for the function of the missing molecule. It is apparent that, while we are beginning to determine the principles that govern the control of neural crest migration, the details of this complex regulatory system are still far from being completely understood.

ACKNOWLEDGEMENTS

The authors are grateful to the British Heart Foundation and the Wellcome Trust for funding their original research in this area.

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