This hybrid was also negative for IGF-II, supporting the results for XER7. These data suggest that IGF-II is localized within the 11pter–11p11 region. Interestingly, the structurally related insulin gene is also localized on the short arm of human chromosome 11 at p15 (ref. 19).

Apart from the insulin and IGF-II genes, the short arm of chromosome 11 also encodes the c-Ha-*ras*1 oncogene and the lactate dehydrogenase A (LDHA) gene[19]. The short arm of chromosome 12 encodes the structurally related genes c-Ki-*ras*2 and the lactate dehydrogenase B (*LDHB*) genes[19]. In the mouse these genes are in conserved groups on chromosomes 7 and 6, respectively[20]. These comparative gene mapping data suggest an apparent common ancestry for these sites.

Deficiencies in IGFs are linked to shortness of stature in humans[7], while their excess is associated with acromegalia[21]. Examination of the structure of these genes in individuals of short stature could indicate the role of IGFs in the aetiology of dwarfism. The recent observations of homologies between the sequences of the B-subunit of platelet-derived growth factor and the protein encoded by the oncogene v-*sis*[22], and the tyrosine kinase domain of the epidermal growth factor receptor and the v-*erb*-B gene product[23] indicate that cellular transformation could result from abnormal expression of a growth factor or its receptor. Moreover, the synthesis of growth factors by some tumours suggests that they may function as autocrine regulators in tumour growth[11]. To date, neither IGF-I and IGF-II nor their receptors have been implicated in the transformation process, although the IGF-I receptor, like the EGF receptor and several oncogenes, has an associated tyrosine kinase activity[24]. The assignment of the IGF genes to human chromosomes 11 and 12 suggests that tumours or hereditary syndromes containing rearrangements and amplification of regions of these chromosomes should be evaluated for abnormal expression of these growth factors. For example, duplication of the p13 to pter region of chromosome 11 has been demonstrated in the Beckwith–Wiedemann syndrome[25], which results in larger birth size, macroglossia and hyperplastic visceromegaly. The features may result from overproduction of IGF-II. The fine structure physical mapping of these genes will allow an assessment of the relationships of IGFs to proto-oncogenes, and to structurally defective sites, on the short arms of chromosomes 11 and 12. The screening of families for the polymorphisms associated with IGF-I will allow us to search for a relation between hereditary defects and this gene, and for close linkage relationships with other genes on this chromosome.

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Segmentation in the vertebrate nervous system

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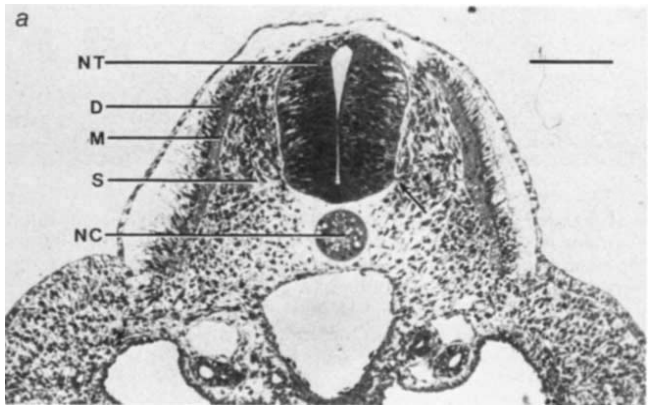
Although there is good evidence that growing axons can be guided by specific cues during the development of the vertebrate peripheral nervous system[1], little is known about the cellular mechanisms involved. We describe here an example where axons make a clear choice between two neighbouring groups of cells. Zinc iodide–osmium tetroxide staining of chick embryos reveals that motor and sensory axons grow from the neural tube region through the anterior (rostral) half of each successive somite. 180° antero-posterior rotation of a portion of the neural tube relative to the somites does not alter this relationship, showing that neural segmentation is not intrinsic to the neural tube. Furthermore, if the somitic mesoderm is rotated 180° about an antero-posterior axis, before somite segmentation, axons grow through the posterior (original anterior) half of each somite. Some difference therefore exists between anterior and posterior cells of the somite, undisturbed by rotation, which determines the position of axon outgrowth. It is widespread among the various vertebrate classes.

In the chick embryo, the outgrowth of both motor and sensory axons occurs in an antero-posterior (rostro-caudal) sequence, those opposite the wing bud appearing between stages 16 and 17 (ref. 2) of embryonic development. By this stage the somite, whose cells growth cones first encounter, has developed into the outer dermatome–myotome (respectively prospective dermis and skeletal muscle) and inner sclerotome (prospective vertebral column). Motor axons leave the neural tube and grow laterally through the sclerotome towards the myotome (Fig. 1*a*); sensory axons appear when the dorsal root ganglion cells, within the sclerotome, send processes both peripherally (to join the motor axons) and centrally towards the dorsal portion of the neural tube. As originally described by Tello[3], and visible in our whole-mount preparations, motor axons precede sensory axons when growing laterally through the sclerotome.

From the start, motor axons grow exclusively through the anterior half of their respective somite (Fig. 1*b*). Because growth cones and their filopodia are visible with the zinc iodide–osmium tetroxide stain (Fig. 1*c*), any axons present in the posterior half are unlikely to have been missed because of their failure to stain. In each of 73 somites examined from 10 stage-17 embryos, motor axonal growth was confined to the anterior half of the somite. In the case of sensory axons, however, processes can be seen occasionally to extend a short distance within the posterior half-somite; 6 such processes were seen in the 73 somites examined. These posterior processes have not been observed during later stages of development, and it is likely that they represent outgrowths from neural crest cells residing in the posterior half-sclerotome which later migrate or degenerate. All motor axons, therefore, and the great majority of sensory axons, grow within the anterior half-somite, specifically its sclerotome portion.

Several questions arise from this observation. First, is the segmented growth of motor axons determined by some intrinsic property of the neural tube, or is it, as the segmented growth of sensory axons suggests, determined by the somite? This question was answered by rotating the neural tube relative to the somitic mesoderm so the neural tube previously opposite the anterior half of the somite became positioned opposite the posterior half. Despite such displacement, in each of 11 such experiments axonal growth remained confined to the anterior half of the somite (Fig. 2). The neural tube is not, therefore, intrinsically segmented with respect to motor axon outgrowth (see also refs 4, 5).

To test whether segmentation instead arises from some property of the somites, the somitic mesoderm was rotated



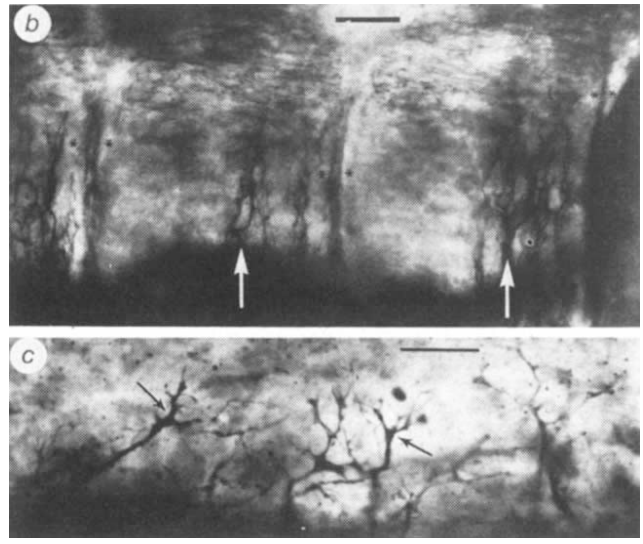


Fig. 1 *a*, Transverse section of a late stage-16 chick embryo, at the level of the wing somites, stained with toluidine blue. The embryo was fixed by immersion in buffered 2.5% glutaraldehyde, plastic-embedded and sectioned at 2 μm. By the stage of motor axon outgrowth (arrow), the somite has developed into dermatome–myotome (D, M) and sclerotome (S). NT, neural tube; NC, notochord. Scale bar, 100 μm. *b*, Late stage-16 embryo, wing region, stained with zinc iodide–osmium tetroxide. The staining procedure was a modification of the method described by Akert and Sandri[23]. The embryos were pinned out on Sylgard dishes and bisected along the antero-posterior axis into left and right halves. They were then immersed in a freshly prepared mixture of 6 ml ZnI_2/1.75 ml of 2% OsO_4, to which had been added a small drop of a concentrated KI_3 solution. The ZnI_2 solution was made by combining 5 g iodine with 15 g powdered zinc in 200 ml water. They were then incubated at 55 °C for 100–105 min, washed with distilled water, dehydrated in alcohols, cleared in xylene and whole-mounted in Permount. Motor axons are seen in the anterior (right in the figure) halves of two somites, having emerged from the neural tube (arrows). The somite borders are enclosed by asterisks. Sensory axons were visible in another (more dorsal) plane of focus. Scale bar, 50 μm. *c*, Whole mount of a stage-21 embryo, wing region, stained with zinc iodide–osmium tetroxide. The end of a spinal nerve is shown, to demonstrate growth cones and their filopodia (arrows). Scale bar, 20 μm.

antero-posteriorly through 180°, leaving the neural tube undisturbed. Strips of segmental plate mesoderm, three to four prospective somites in length, were removed from donor embryos and implanted in host embryos, opposite the wing bud, after rotation (Fig. 3). Eight such operations were performed, in six of which the medio-lateral axis was also reversed. In all eight cases, axonal growth was now confined to the posterior (original anterior) halves of the grafted somites. Both anterior and posterior to the graft, axonal growth was, as expected, through the anterior halves of the host somites. In five control experiments where the segmental plate mesoderm was removed and replaced with the normal orientation, and in two where only the medio-lateral axis of the segmental plate was reversed, axons were positioned as in unoperated embryos. These experiments show that neural segmentation results from the somites. They also show that the anterior–posterior difference must be determined at or before somite segmentation from the segmental plate, and that the embryonic axis is unable to regulate for its positional disturbance. Furthermore, rotation about the medio-lateral axis is without effect on the segmentation of neural outgrowth.

What properties of the somite might restrict axonal growth to its anterior half? As axons first encounter sclerotome cells (Fig. 1*a*), there is presumably a difference between those cells in the anterior half-sclerotome and those in the posterior half-sclerotome; anterior cells permit axonal growth and/or posterior cells inhibit it. One possibility would be that motor axons emerge simultaneously opposite both sclerotome halves, but can only grow out through anterior sclerotome. We might then expect to see axons in the posterior region growing along the surface of the neural tube towards the anterior half-sclerotome. However, we do not (Fig. 4*a*). The first axons emerge only from neural tube opposite the anterior half-sclerotomes; later axons, though, do emerge opposite the posterior half-sclerotomes and then turn towards the anterior half-sclerotome on either side (Fig. 4*b*). Chick motor axons grow laterally from their cell bodies so as to leave the neural tube at the same antero-posterior level as that at which they arise[6]. Therefore, and contrary to Tello's

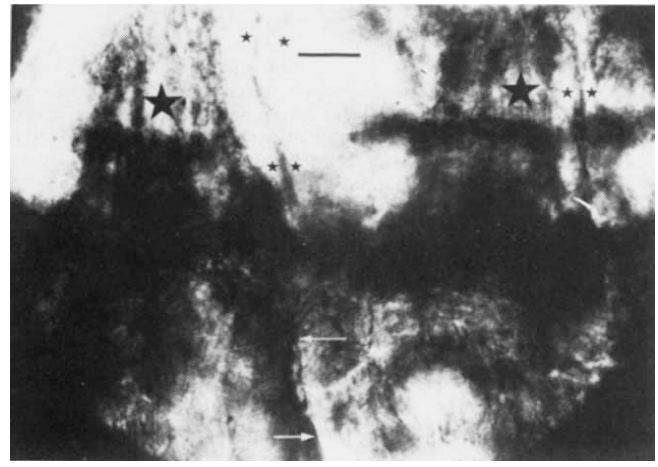


Fig. 2 Neural tube rotation: 11 host embryos were incubated at 38 °C to stage 11–13. Donor embryos were incubated to the same developmental stage and then pinned out in Sylgard dishes containing 0.1% trypsin (Difco) in calcium- and magnesium-free Tyrode's solution at room temperature. Within 3 min the neural tube could be removed, free of ectoderm, endoderm and notochord. Lengths of neural tube opposite two, three or four somites were cut from the presumptive wing region and their dorsal–anterior portions marked with carmine particles. Neural tube of the same length and position was removed from host embryos *in ovo* with fine tungsten needles, leaving the notochord and somites intact. The donor grafts were then implanted in the host embryos, after 180° reversal about the antero-posterior axis. In this way, neural tube previously opposite an anterior half-somite came to lie opposite the posterior half. After 2 days' further incubation *in ovo*, the embryos were stained and examined as described for Fig. 1*b*. In the example shown, the junction between donor and host neural tube is arrowed: axons (large asterisks) grow through anterior (right) halves of somites from both donor (left) and host (right) neural tubes. Small asterisks enclose somite borders. Scale bar, 50 μm.

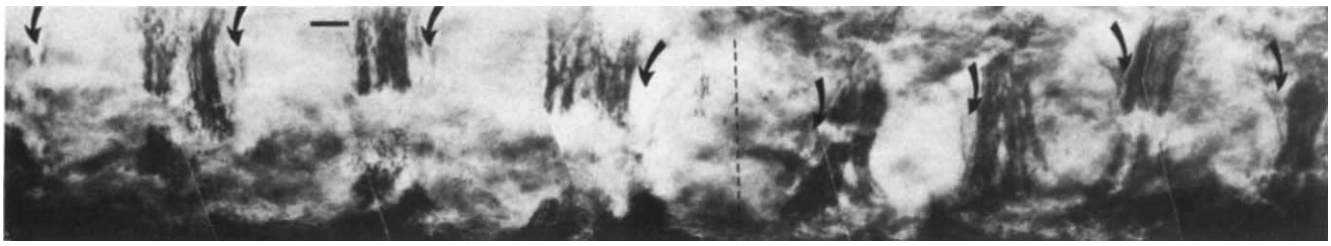


Fig. 3 Rotation of somitic mesoderm; technique as Fig. 2 legend. Lengths of segmental plate mesoderm, two to four prospective somites in length, were cut from the wing region of donor embryos and marked with carmine at one end. Similar lengths of segmental plate mesoderm were removed, together with overlying ectoderm, from the wing region of the same or opposite side of host embryos, leaving neural tube and endoderm intact. The grafts were then implanted in the hosts after 180° reversal about the antero-posterior axis or both antero-posterior and medio-lateral axes. Following 2 days' further incubation *in ovo*, the embryos were examined as for Fig. 1*b*. In the photomontage the neural tube is inferior. The dashed line separates the host region (left, posterior) from the graft region (right, anterior). Arrows mark the somite borders. In the graft region axons are now immediately anterior to the somite borders. Scale bar, 50 μm.

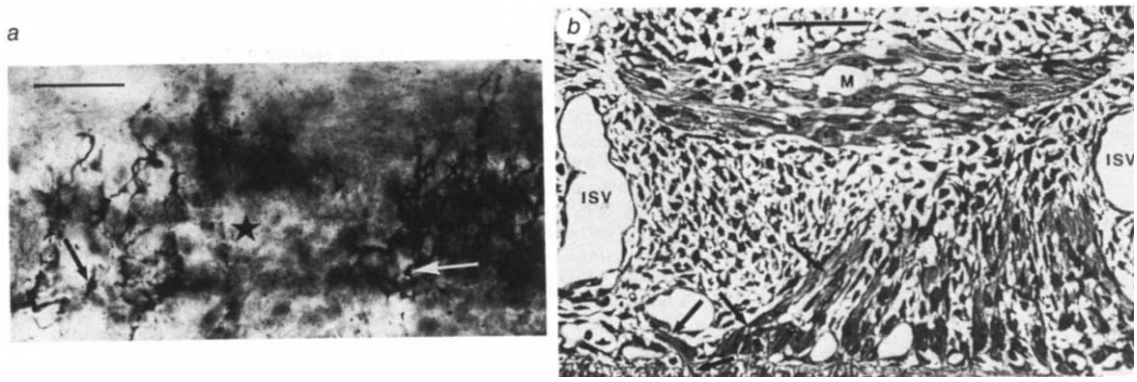


Fig. 4 *a*, Whole mount of neural tube and sclerotome of a late stage-16 embryo, wing region, stained with zinc iodide–osmium tetroxide. After staining, the neural tube was bisected along its long axis and most of the somitic mesoderm cut off laterally. The medial portions of two sclerotomes are left adjacent to the neural tube. Motor axons leave the ventral surface of the neural tube (arrows) and grow (initially dorsolaterally) in the anterior half of each sclerotome. No axons are seen near the surface of the neural tube opposite the posterior half of the sclerotome (asterisk), the same being true for adjacent planes of focus. Scale bar, 50 μm. *b*, Longitudinal section of a stage-21 embryo, prepared as for Fig. 1*a*, stained with toluidine blue. Neural tube inferior (asterisks); anterior to the right. Axons emerging opposite posterior half-sclerotome (arrows) are seen, directed either anteriorly or posteriorly towards, respectively, the anterior half of the same somite or the anterior half of the adjacent (posterior) somite. The myotome (M) is seen straddling the intersegmental blood vessels (ISV). Scale bar, 50 μm.

description[3], the extension of motor axons occurs in a discontinuous punctuated manner along the neural tube, being first from cell bodies opposite the anterior half-sclerotomes. Only later do cells opposite the posterior half-sclerotomes extend axons; and these axons presumably fasciculate on the previous 'pioneer' outgrowths, after penetrating the basal lamina surrounding the neural tube. If axon extension is initiated as a consequence of some influence of the anterior half-sclerotome cells, this influence is presumably not an absolute requirement for axon extension, for prior destruction of the somitic mesoderm, at least at the stage of somite segmentation, does not prevent axon outgrowth[5].

Since Remak's[7] original observations on the development of the vertebrate column, there have been many further descriptive studies (see refs 8–10 for reviews). It has often been noted that the sclerotome subdivides into anterior and posterior regions of, respectively, lesser and greater cell densities, a difference which arises after the first axonal outgrowths (unpublished observations). Remak also saw that the spinal nerve and ganglion develop in the anterior half-sclerotome. Von Ebner[11] described a relatively cell-free space or fissure between anterior and posterior sclerotome halves in both snake and chick embryos, a feature which has been variously confirmed[8] or refuted as a fixation artefact[9]. We confirm its existence in the chick embryo, finding it visible in the presence or absence of fixation. Axons have never been seen to cross this boundary between anterior and posterior sclerotome halves, so it is likely that the fissure also represents the boundary between anterior and posterior portions of sclerotome with respect to axon growth.

Detwiler's[4] classical experiments on neural segmentation in the axolotl showed that neural segmentation in this species results from mesodermal segmentation. Could, then, an antero-posterior subdivision of the sclerotome determine neural segmentation in all vertebrates? Certainly anterior–posterior sclerotome cell density differences have been described in all vertebrate classes[7–9,12–17], and the spinal nerve is seen in the anterior half-sclerotome. What is critical, however, is the timing of appearance of the sclerotome changes in relation to axon outgrowth. In the case of teleost[18] and elasmobranch[19] fishes, and anuran amphibia[20,21], where studied, the first motor axons grow out at a stage when there may be few or no sclerotome cells intervening between neural tube and myotome. In these conditions the myotome cells alone may determine segmentation. Nevertheless, sclerotome cells have been described in association with axons[19,21], and such myotomal determination of neural segmentation does not easily explain the coincident segmentation of sensory axons. Furthermore, in at least one elasmobranch fish, *Scyllium catulus*, there is a sclerotome-filled space, several cell diameters wide, between neural tube and myotome at the stage that ventral root axons first appear (ref. 22 and our own unpublished observations). Like *S. catulus* and

the chick, in apodan amphibia[13], reptiles[15], other birds[16] and mammals[17], motor and sensory axons probably first grow out at a stage when sclerotome cells have accumulated between neural tube and myotome (Fig. 1*a*). Therefore, it is presumably towards this population of cells that we should look for an explanation of neural segmentation in these vertebrates. The experiments described here have shown that both motor and sensory axons preferentially associate with cells of the anterior half-sclerotome. How anterior and posterior cells differ in terms of cell lineage and molecular properties remains to be determined.

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Immune sera recognize on erythrocytes a *Plasmodium falciparum* antigen composed of repeated amino acid sequences

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Protective immune responses against the asexual stages of the human malaria parasite, *Plasmodium falciparum*, are most probably directed against exposed antigenic determinants on the surface of the free merozoite[1–3] or the infected red blood cell[4], and therefore antigens in these locations are candidates for testing as components of a defined molecular vaccine. To facilitate the search for such antigens, we recently developed a method for the expression of *P. falciparum* proteins in *Escherichia coli* as fused polypeptides[5]. Many clones producing antigens were detected by screening with immune human sera[6,7]. We show here that antibodies against the fused polypeptide expressed by one such clone react with a *P. falciparum* protein that is synthesized late in schizogony and is later present on the surface of the ring-infected erythrocyte. The protein is composed of repeating subunits of 8, 4 and 3 amino acids and is present in all isolates of *P. falciparum* examined.

The construction of a cDNA library of *P. falciparum* in the vector λgt11-Amp3 has been described previously[5,8]. cDNA was inserted into the unique *Eco*RI site near the C-terminus of β-galactosidase, resulting in expression of fused polypeptides in *E. coli*. Clones expressing fused polypeptides that correspond to natural antigens were detected by an *in situ* immunoassay using immune human sera. One clone, designated Ag13, produced a large fused polypeptide with a molecular weight (MW) of 156,000 (156 K) which was presumably composed of 116K of β-galactosidase sequence and 40K of *P. falciparum* sequence[5]. Immunoblot analyses using anti-β-galactosidase antibodies and anti-*P. falciparum* antibodies confirmed that the fused polypeptide contained antigenic determinants recognized by both sera[5,7]. Mice and rabbits were immunized with lysates of induced bacteria or with the fused polypeptide purified as described previously[9].

The *P. falciparum* antigen corresponding to the Ag13 fragment was identified as an acidic protein of 155K MW, by immunoprecipitation of biosynthetically-labelled asynchronous

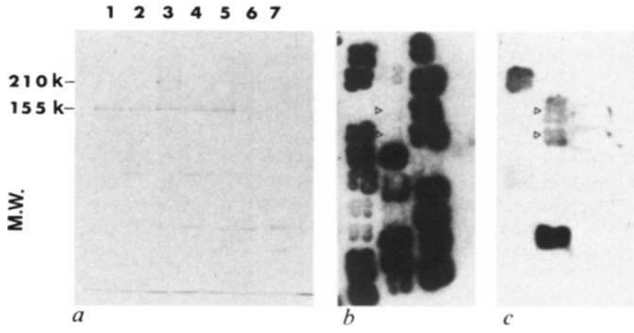

Fig. 1 Identification of the native protein corresponding to Ag13. *a*, One-dimensional SDS-polyacrylamide gels of immunoprecipitates *b*, *c*, Hybridization of stage-specific *P. falciparum* cDNA to antigen-positive clones.
Methods: *a*, Parasites of the FC27 (ref. 18) line were grown in asynchronous culture *in vitro* according to the method of Trager and Jensen[19]. At a parasitaemia of ~3% the parasites were transferred to a methionine-free medium supplemented with ^{35}S-methionine for 18 h. Parasites were collected by centrifugation and lysed in 0.5% Triton X-100, 150 mM NaCl, 10 mM EDTA and 5 mM Tris *p*H 8. Aliquots of labelled parasite lysate were reacted with various sera. Formalin-fixed *Staphylococcus aureus* was used as a solid-phase reagent. Immunoprecipitates were prepared and analysed by one-dimensional SDS–polyacrylamide gel electrophoresis as described previously[20]. Gels were fluorographed. The antisera were prepared by injecting mice with lysates[21] of Ag13 or five independent antigen-positive clones (Ag24,28,32,46,47) producing fragments of the same molecule[6,22]. These clones were identified by cross-hybridization with the Ag13 cDNA[6] and by positive signal on the colony immunoassay with anti-Ag13 antibodies[22]. All sera recognized a 155K MW molecule but some also recognized a 210K MW molecule. Lane 1, anti-Ag28; lane 2, anti-Ag13; lane 3, anti-Ag24; lane 4, anti-Ag46; lane 5, anti-Ag47; lane 6, anti-Ag32; lane 7, anti-λAmp3 vector. *b*, *c*, Parasites of the FC27 line[18] were synchronized twice by treatment with 5% sorbitol according to the method of Lambros and Vanderberg[23] as modified by Freeman and Holder[24]. The parasites were cultured *in vitro*[18] for one cycle of growth and merozoites collected according to the method of Freeman and Holder[25]. The merozoite preparation was stained with 10% Giemsa and examined under light microscopy and the degree of contamination by other life cycle forms was less than 1 infected cell per 1,000 merozoites. In a separate synchronization experiment parasites were collected at the transition point between mature trophozoites and early schizonts. No schizonts with greater than four nuclei were seen by light microscopy and trophozoites constituted over 75% of the parasites present. The parasite preparations were solubilized in 6 M guanidine hydrochloride in 0.4 M sodium acetate *p*H 5.2 and RNA was prepared as described elsewhere[5]. Aliquots of total RNA from the two preparations were transcribed into labelled cDNA and 3×10^6 c.p.m. per ml of each were hybridized to prepared nitrocellulose filters[26]. The nitrocellulose filter was a replica of an array of antigen-positive clones including Ag13 sequences (arrowed). In *b* the array was reacted with cDNA prepared from the trophozoite plus early schizont preparation while in *c* the array was reacted with cDNA from free merozoites. The Ag13 sequences are strongly recognized by the merozoite cDNA preparation.