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Expression of the trk Family of Neurotrophin Receptors in Developing and Adult Dorsal Root Ganglion Neurons

Heidi S. Phillips and Mark P. Armanini

Phil. Trans. R. Soc. Lond. B 1996 **351**, 413-416
doi: 10.1098/rstb.1996.0036

Expression of the trk family of neurotrophin receptors in developing and adult dorsal root ganglion neurons

HEIDI S. PHILLIPS AND MARK P. ARMANINI

Department of Neuroscience, Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, California 94080, U.S.A.

SUMMARY

Expression of trk receptors is a major determinant of neurotrophin responsiveness of sensory neurons. Although it has been apparent for some time that subpopulations of dorsal root and trigeminal ganglion neurons respond *in vitro* to each of the members of the neurotrophin family, the extent to which functionally distinct subclasses of sensory neurons are dependent on the actions of different neurotrophins for their development and function remains an active area of investigation. One step towards elucidating the role of various neurotrophins in development and function of sensory neurons has been to examine the distribution of trk receptors on sensory neurons. These studies have clearly revealed that members of the trk family are differentially expressed in functionally distinct populations of both developing and mature sensory neurons and, further, have provided evidence consistent with a shift in neurotrophin responsiveness during the development of sensory neurons.

1. TRK EXPRESSION IN DEVELOPING DRG CELLS

Examination of trk expression in dorsal root ganglia (DRG) from the cervical region of mouse embryos indicates that trkA, B, and C are differentially regulated during development (figures 1 & 2). At E10.5, the earliest time examined, trkC is strongly expressed by the majority of cells within the ganglia, whereas trkB is present in a patchy distribution, and trkA is present at low levels. From E10.5 to 13.5, trkA expression is markedly upregulated to appear in the majority of cells, whereas trkC expression undergoes a concomitant restriction such that, at E13.5, clear hybridization for trkC is seen in a subpopulation of cells which represent a minority of DRG neurons. During the same period of time, trkB expression is seen in a modest subpopulation of cells, and the extent and level of expression within the ganglia undergoes no obvious changes. From E13.5 until birth, each of the trks is clearly expressed in a subpopulation of DRG neurons and, although cell counts have not been performed, it is clear that a majority of DRG neurons express trkA and that far fewer cells express trkB or trkC.

The factors responsible for regulating trk expression during development are largely unknown. The time course of trkA upregulation in developing mouse DRG seen with in situ hybridization (figure 1) corresponds quite well with the profile of trkA expression in mouse trigeminal ganglia, as quantified by RT-PCR (reverse transciptase polymerase chain reaction) (Wyatt & Davies 1993). Given the close temporal correspondence between trkA upregulation and peripheral target innervation, it would seem that the marked increase in trkA expression might be driven by the exposure of sensory neurons to target-derived NGF. This hypothesis does not, however, appear to be correct, as the profile of trkA expression is normal in trigeminal ganglia from mice homozygous for a targeted disruption in the NGF gene (Davies *et al.* 1995). The influence of other target-derived factors on upregulation of trkA expression has not been examined, nor have any studies addressed the factors contributing to the concomitant downregulation of trkC expression.

The early expression of trkC in mouse DRG is consistent with the ability of NT3 to promote survival *in vitro* of neurons from early mouse trigeminal ganglion (Buchman & Davies 1993; and for a review see Davies 1994). Early expression of trkC has been reported in avian species, where expression on migrating neural crest cells and in the earliest stages of gangliogenesis correlates nicely with the ability of NT3 to influence proliferation, differentiation, and/or survival of precursors (Pinco *et al.* 1993; Gaese *et al.* 1994; Kahane & Kalcheim 1994; Zhang *et al.* 1994; Williams *et al.* 1995). Although it is tempting to speculate that the expression of trkC during early development of DRG neurons is related to early actions of NT3, the significantly milder phenotype of trkC-deficient mice as compared to NT3-deficient mice, suggests that some actions of NT3 may not be mediated via trkC (Fariñas *et al.* 1994; Ernfors *et al.* 1994*b*; Klein *et al.* 1994). In this regard, it is important to note that the studies of trkC localization in developing sensory neurons have employed probes which do not distinguish between truncated and catalytic isoforms of the receptor.

The shift in expression from a predominance of trkC in the E10.5 mouse cervical DRG to a predominance of trkA in the E13.5 ganglia nicely parallels the shift in responsiveness for survival *in vitro* of mouse trigeminal neurons from BDNF or NT3 to NGF (see Davies 1994). The widespread expression of trkC in early ganglion development followed by progressive restriction to a subpopulation of cells has been reported in avian systems, where expression at later points in

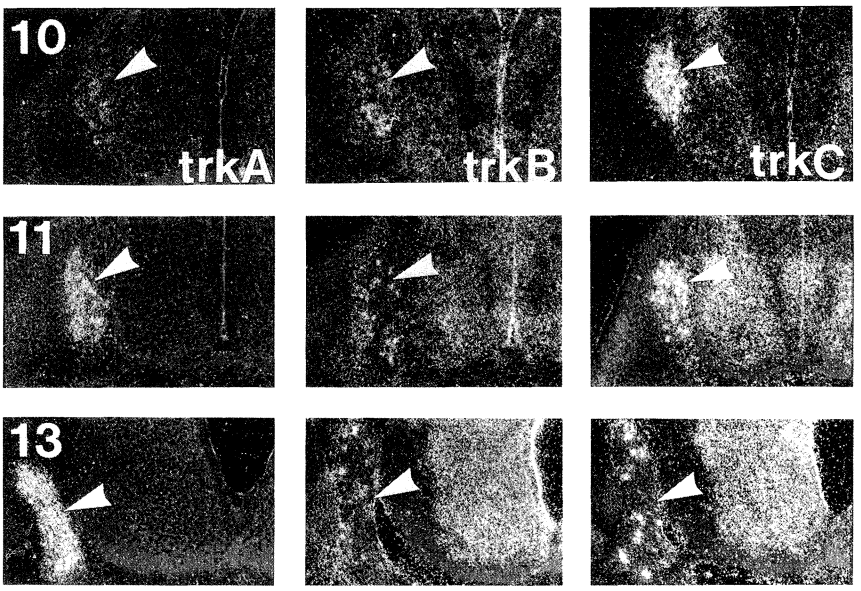

Figure 1. Darkfield micrographs of dorsal root ganglia from the cervical region of embryonic or adult mice hybridized with probes to the extracellular domain of trkA, trkB, or trkC (as labelled). Panels labelled 10, 11, 13 display transverse sections through immerse-fixed embryos at 10.5, 11.5, 13.5 days of gestation. Arrows indicate position of the ganglia.

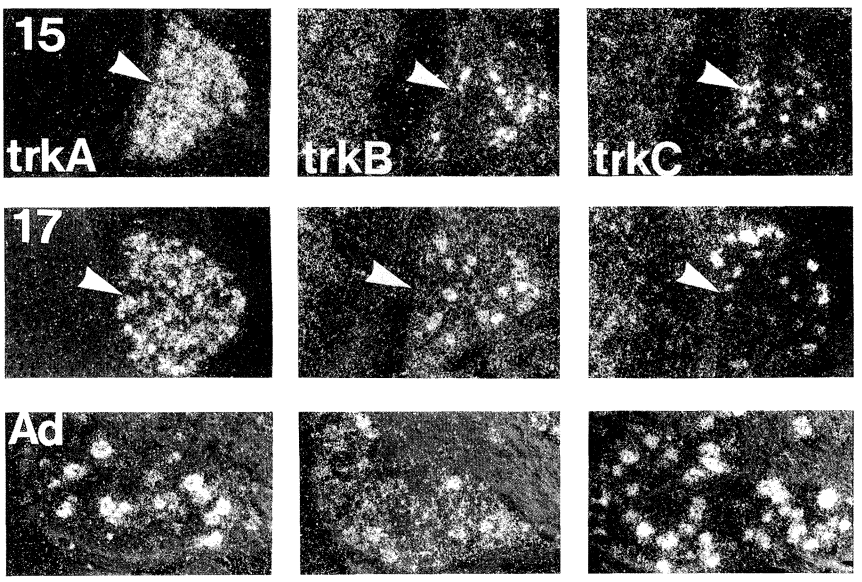

Figure 2. Darkfield micrographs of dorsal root ganglia from the cervical region of embryonic or adult mice hybridized with probes to the extracellular domain of trkA, trkB, or trkC (as labelled). Panels labelled 15, and 17, display transverse sections through immerse-fixed embryos at 15.5, and 17.5 days of gestation. Arrows indicate position of the ganglia. Panels marked Ad demonstrate sections through ganglia from adult animals.

development is maintained predominantly in the large cells of the lateroventral region (Kahane & Kalcheim 1994; Zhang *et al.* 1994; Williams *et al.* 1995). Such findings are consistent with the demonstration that NT3 promotes survival of developing chick muscle sensory neurons in culture (Hory-Lee *et al.* 1993). Although the lack of obvious change in trkB distribution seen in dorsal root ganglia does not parallel the loss of responsiveness of trigeminal neurons to BDNF, it is possible that shifts in the proportions of truncated and catalytic isoforms of trkB might contribute to changes in responsiveness.

The qualitative appearance of trkA, trkB, and trkC-expressing mouse DRG neurons from E13.5 through birth (figure 1) or in developing rat (Ernfors *et al.* 1993; Mu *et al.* 1993) is consistent with much of the information derived from recent gene targeting (for review see Snider 1994). Gene targeting studies have indicated that small diameter DRG neurons are critically dependent for survival during embryonic development on NGF and its receptor trkA, whereas proprioceptive afferents are critically dependent on NT3 and trkC (Crowley *et al.* 1994; Ernfors *et al.* 1994; Fariñas *et al.* 1994; Klein *et al.* 1994; Smeyne *et al.* 1994; Tessarollo *et al.* 1994). Consistent with the predominance of small diameter afferents within normal DRG, both trkA- and NGF-deficient mice display 70–80% fewer L4/5 DRG neurons than do

their wild-type littermates. This number matches nicely that observed rat pups treated *in utero* with antibody specific to NGF (Ruit *et al.* 1992). In keeping with the finding that a smaller number of cells express trkB or trkC as compared to trkA during mid to late embryogenesis of both rat and mouse (figure 1; see Ernfors *et al.* 1993; Mu *et al.* 1993), both trkB- and trkC- deficient mice and mice lacking BDNF, NT3, or NT4/5 lack fewer DRG neurons than do NGF- or trkA-deficient mice (Conover *et al.* 1992; Klein *et al.* 1993; Ernfors *et al* 1994*a*,*b*; Jones *et al.* 1994; Klein *et al.* 1994; Liu *et al.* 1995). Further, the loss of proprioceptive afferents in trkC knockout mice, are nicely supported by the observations of Mu *et al.* (1993) that expression of trkC in mid- to late-embryonic rat DRG is confined to large cells.

Taken together, the distribution of trk receptors and the gene deletion studies indicate that, subsequent to the period of peripheral target innervation, the populations of DRG neurons expressing trkA and trkC are largely non-overlapping. The normal appearance and subsequent loss of trkA-expressing cells in NGF-deficient mice and of trkC-expressing neurons in NT3-deficient mice argues against redundant trophic influences of NGF and NT3 on neurons coexpressing trkA and trkC (Crowley *et al* 1994; Tessarollo *et al* 1994; Davies *et al* 1995). Further, these findings strongly suggest that all cells which express trkA during mid to late embryonic development are dependent on NGF for survival during embryonic life, whereas all cells expressing trkC during the same period of time are dependent on NT3.

2. TRK EXPRESSION IN ADULT DRG NEURONS

Although an increasing body of evidence supports the concept that distinct subpopulations of primary sensory neurons require different target-derived neurotrophins for survival during embryonic life (see Snider 1994), the extent to which functionally distinct subpopulations of adult sensory neurons are similarly responsive to the actions of different members of the neurotrophin family remains to be established. The distribution of trk receptors on adult rat DRG is currently an active area of investigation and has revealed that trk expression correlates with a variety of parameters associated with functional classification. Specifically, trk expression varies among subpopulations of adult DRG neurons identified by cell size, class of peripheral target, and expression of neurochemical makers.

As in developing dorsal root ganglia, cell size histograms of adult rat ganglia indicate selective expression of trkA and trkC on small to medium vs. large neurons, respectively (Mu *et al.* 1993; McMahon *et al.* 1994). Consistent with the dependence of proprioceptive neurons on NT3 and trkC for survival during development (Hory-Lee *et al* 1993; Fariñas *et al.* 1994; Klein *et al* 1994; Tessarollo *et al.* 1994), in the adult rat, nearly 75% of DRG neurons projecting through the gastrocnemius nerve express mRNA encoding for the catalytic isoform of trkC (McMahon *et al.* 1994). In marked contrast, only a minority of muscle afferents express trkA (20%), and most of these represent small diameter afferents. In keeping with the selective dependence of small, nociceptive neurons on trkA or NGF for survival during embryogenesis, many small cells with cutaneous targets and nearly all visceral afferents projecting through the pelvic nerve express trkA (McMahon *et al.* 1994).

Interestingly, some cells which are dependent on NGF for survival during development do not express trkA in maturity. In contrast to the 70–80% of cells which require NGF for survival during embryogenesis, only 40–45% of adult DRG neurons express trkA (Verge *et al.* 1992; McMahon *et al.* 1994; Snider 1994). Within adult ganglia, cells at the smallest end of the size spectrum do not express trkA, and in fact, do not express detectable signal for any of the known trks (McMahon *et al.* 1994). Recent work by Averill *et al.* 1995 indicates that the trkA-expressing population of cells in the adult corresponds nearly perfectly to the population of cells expressing CGRP, whereas the vast majority of non-trk expressing cells may be labelled by marker IB4.

Although all available information supports the conclusion that trkA and trkC are expressed on largely non-overlapping populations of adult DRG neurons, the profile of trkB expression is less clear. Estimates of the percentages of adult rat DRG neurons expressing trkB vary significantly, ranging from 10% (Wright & Snider 1995) to 26–27% (McMahon *et al.* 1994; Wetmore and Olson 1995). Consistent with the higher estimates, (Acheson *et al.* 1995) have demonstrated that as many as 35% of adult DRG neurons are dependent on autocrine or paracrine actions of BDNF for promoting survival *in vitro*. Studies differ not only on the percentages of trkB-expressing cells, but also as to whether trkB-expressing neurons are distinct from those which express trkA and trkC (McMahon *et al.* 1994; Wright & Snider 1995). One possible explanation is that a small percentage of cells express trkB at high levels and do not express other trks, whereas a larger number of cells express trkB at lower levels along with either trkA or trkC. Coexpression of trkB with either trkA or trkC might allow an individual sensory neuron to respond to both local actions of BDNF as well as to target-derived influences of NGF and NT3. The possibility that a small subpopulation of DRG neurons responds to target-derived influences of BDNF or NT4/5 deserves further attention.

3. CONCLUSION

The pattern of trk distribution during development of dorsal root ganglia is consistent with the increasing body of evidence which supports the concept that distinct subpopulations of primary sensory neurons require different target-derived neurotrophins for survival during embryonic life. Shifts in expression of trkA and trkC in early DRG development correlate well with the shift in survival responses of trigeminal ganglion neurons *in vitro*, but the extent to which individual sensory neurons respond to sequential actions of multiple neurotrophins during development

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remains to be established. Further, the potential role of truncated isoforms of trkB and/or trkC in modulating responsiveness of developing sensory neurons has yet to be examined.

In adult ganglia, trk expression correlates well with markers of functionally distinct subpopulations of sensory neurons, indicating the potential for NGF to influence function of small diameter visceral and cutaneous afferents via trkA , and that of NT3 to influence large diameter cutaneous and muscle afferents via trkC. The extent to which trkB-expressing DRG neurons constitute a distinct subpopulation of sensory neurons remains a topic for further investigation.

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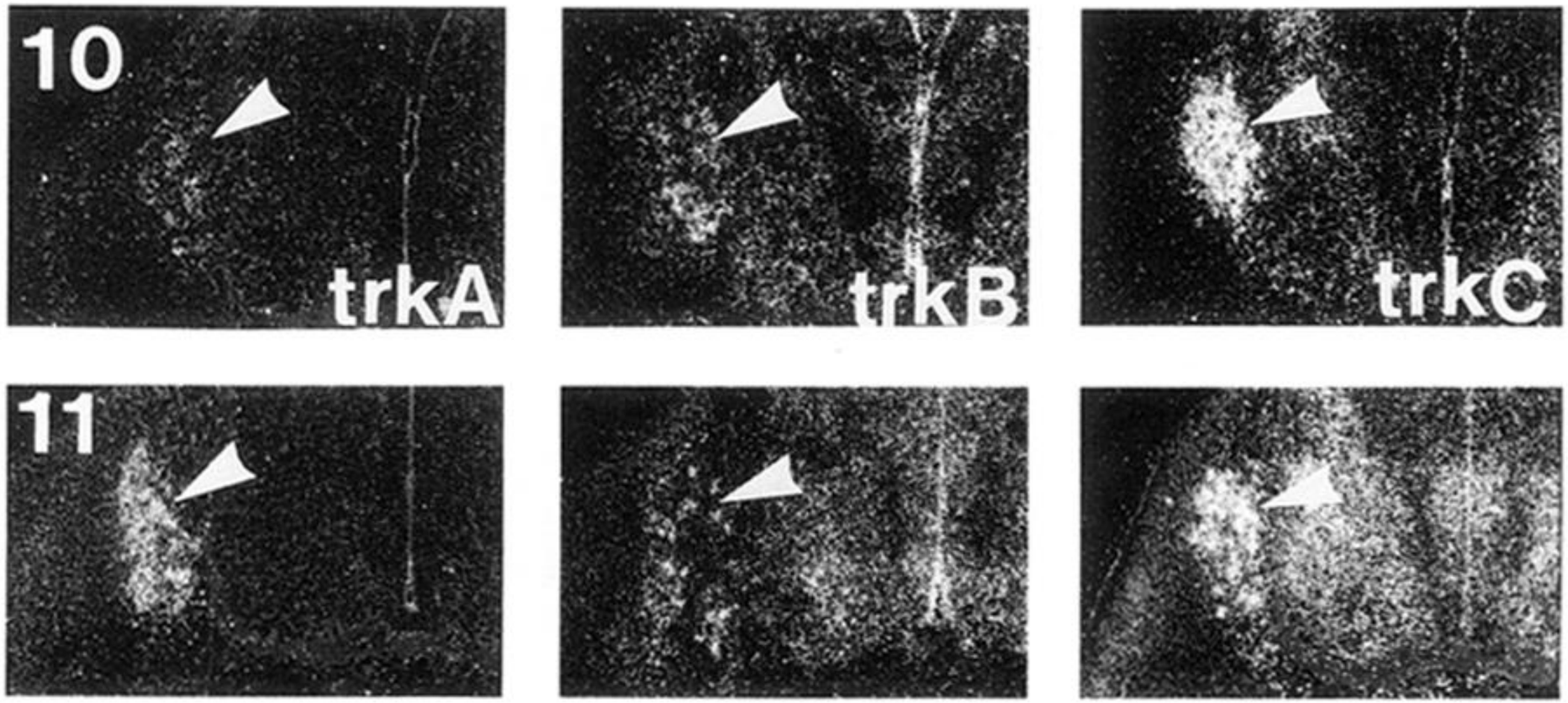

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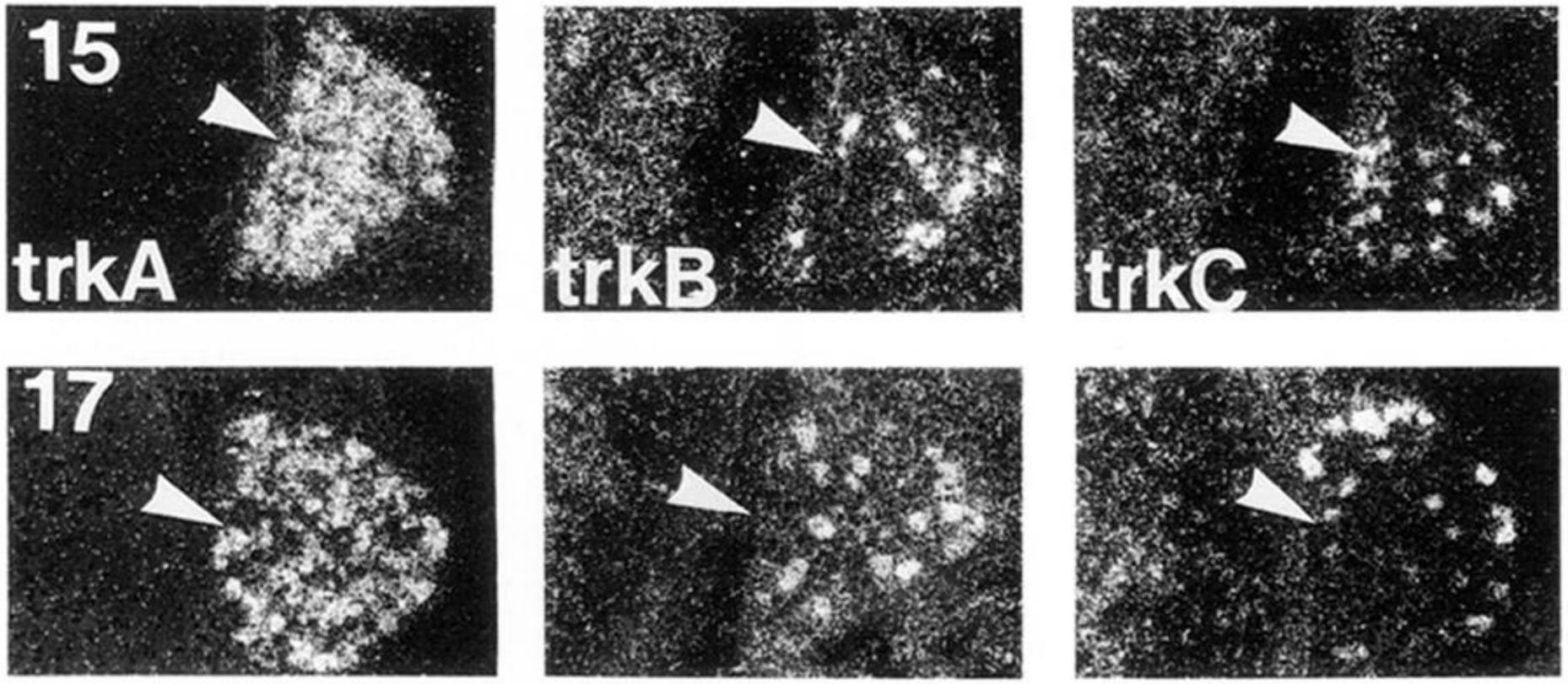

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