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The transcription factor chicken *Scratch2* is expressed in a subset of early postmitotic neural progenitors

Felipe Monteleone Vieceli[a], Marcos Simões-Costa[b], José Antonio Turri[a], Tatiane Kanno[a], Marianne Bronner[b], Chao Yun Irene Yan[a,*]

[a] *Department of Cell and Developmental Biology, Universidade de São Paulo, São Paulo, SP, Brazil*
[b] *Division of Biology, California Institute of Technology, Pasadena, CA, USA*

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ABSTRACT

Scratch proteins are members of the Snail superfamily which have been shown to regulate invertebrate neural development. However, in vertebrates, little is known about the function of Scratch or its relationship to other neural transcription factors. We report the cloning of chicken *Scratch2* (*cScrt2*) and describe its expression pattern in the chick embryo from HH15 through HH29. *cScrt2* was detected in cranial ganglia, the nasal placode and neural tube. At all stages examined, *cScrt2* expression is only detected within a subregion of the intermediate zone of the neural tube. *cScrt2* is also expressed in the developing dorsal root ganglia from HH22–23 onwards and becomes limited to its dorsal medial domain at HH29. phospho-Histone H3 and BrdU-labeling revealed that the *cScrt2* expression domain is located immediately external to the proliferative region. In contrast, *cScrt2* domain overlapped almost completely with that of the postmitotic neural transcription factor *NeuroM/Ath3/NEUROD4*. Together, these data define *cScrt2*-positive cells as a subset of immediately postmitotic neural progenitors. Previous data has shown that Scrt2 is a repressor of E-box-driven transcription whereas NeuroM is an E-box-transactivator. In light of these data, the co-localization detected here suggests that Scrt2 and NeuroM may have opposing roles during definition of neural subtypes.

The Scratch family is part of the Snail superfamily of zinc finger transcription factors. In vertebrates, these possess characteristic DNA-binding zinc finger motifs at the C-terminus and a basic amino acid-rich domain (SNAG domain) at the N-terminus. In addition, Scratch proteins also have a conserved Scratch domain that is not found in the other members of the Snail superfamily (Manzanares et al., 2001).

Accumulating evidence from expression patterns and loss and gain of function studies suggest that Scratch proteins have a conserved role in promoting neural fate across several phyla (Ellis and Horvitz, 1991; Roark et al., 1995; Nakakura et al., 2001b; Marín and Nieto, 2006; Rodríguez-Aznar and Nieto, 2011). In the fly embryo, ectopic expression of scratch (scrt) produces extra neurons and represses the expression of non-neural genes (Roark et al., 1995). However, deletion of *scrt* alone results in a very mild ocular phenotype. A significant effect was only seen when *scrt* was eliminated in conjunction with the pan-neural bHLH transcription factor *dpn* (*deadpan*). Similarly, in the nematode *Caenorharbditis elegans*, gain of function of the homologue of scrt (CES-1) was shown to prevent apoptosis of neuronal precursors but loss of function of CES-1 alone failed to generate an obvious phenotype (Ellis and Horvitz, 1991). Together, these data indicate that Scratch function in neural development is intertwined with other nuclear elements and that it can regulate bHLH transcription factor activity. In support of this, CES-1 represses the expression of pro-apoptotic genes through competition with bHLH heterodimers (Thellmann et al., 2003). Human SCRT1 also competes with bHLH transcription factors in binding to E-box motifs (Nakakura et al., 2001a; Paul et al., 2012). Finally, in the vertebrate embryo *Scrt1/2* have been consistently associated with postmitotic neural progenitors (Nakakura et al., 2001a; Marín and Nieto, 2006; Rodríguez-Aznar and Nieto, 2011).

In contrast to invertebrates, less is known in vertebrates about the expression domain of *Scratch* genes relative to other established neural differentiation transcription factors. Considering that neural differentiation occurs concomitantly with migration towards external layers of the developing nervous system (Leber and Sanes, 1995), the anatomical position of a particular gene's expression domain relative to others, whose function are known, contributes to refining its functional position in the neural differentiation hierarchy. The match between gene function and expression domain is clearer in the posterior neural tube, whose early anatomy is significantly simpler than the cortex (Diez del Corral and Storey, 2001).

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* Corresponding author. Tel.: +55 11 3091 7742; fax: +55 11 3091 7402.
E-mail address: ireneyan@usp.br (C.Y.I. Yan).

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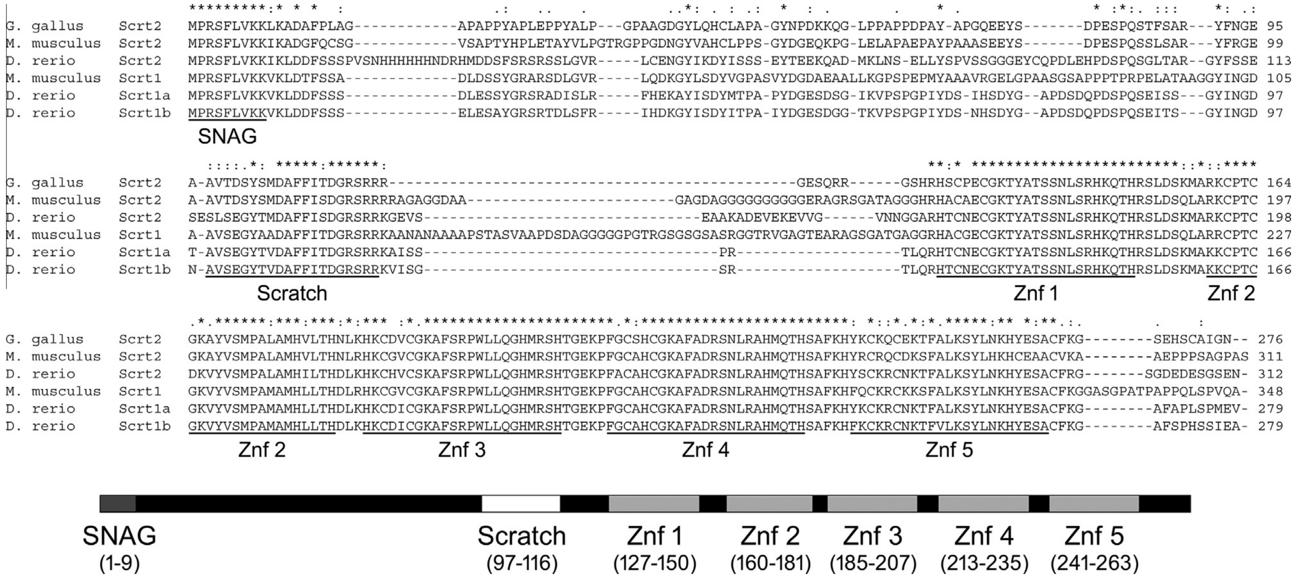

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G. gallus     Scrt2    MPRSFLVKKLKADAFPLAG------------APAPPYAPLEPPYALP---GPAAGDGYLQHCLAPA-GYNPDKKQG-LPPAPPDPAY-APGQEEYS-------DPESPQSTFSAR---YFNGE 95
M. musculus   Scrt2    MPRSFLVKKIKADGFQCSG------------VSAPTYHPLETAYVLPGTRGPPGDNGYVAHCLPPS-GYDGEQKPG-LELAPAEPAYPAAASEEYS-------DPESPQSSLSAR---YFRGE 99
D. rerio      Scrt2    MPRSFLVKKIKLDDFSSSPVSNHHHHHHNDRHMDDSFSRSRSSLGVR-----LCENGYIKDYISSS-EYTEEKQAD-MKLNS-ELLYSPVSSGGGEYCQPDLEHPDSPQSGLTAR--GYFSSE 113
M. musculus   Scrt1    MPRSFLVKKVKLDTFSSA-------------DLDSSYGRARSDLGVR-----LQDKGYLSDYVGPASVYDGDAEAALLKGPSPEPMYAAAVRGELGPAASGSAPPPTPRPELATAAGGYINGD 105
D. rerio      Scrt1a   MPRSFLVKKVKLDDFSSS-------------DLESSYGRSRADISLR-----FHEKAYISDYMTPA-PYDGESDSG-IKVPSPGPIYDS-IHSDYG--APDSDQPDSPQSEISS---GYINGD 97
D. rerio      Scrt1b   MPRSFLVKKVKLDDFSSS-------------ELESAYGRSRTDLSFR-----IHDKGYISDYITPA-IYDGESDGG-TKVPSPGPIYDS-NHSDYG--APDSDQPDSPQSEITS---GYINGD 97

                         ::::.*: *****:******:                                                 **:* ***********************::*::****
G. gallus     Scrt2    A-AVTDSYSMDAFFITDGRSRRR-------------------------------------------GESQRR------GSHRHSCPECGKTYATSSNLSRHKQTHRSLDSKMARKCPTC 164
M. musculus   Scrt2    A-AVTDSYSMDAFFISDGRSRRRRAGAGGDAA------------------------GAGDAGGGGGGGGGERAGRSGATAGGGHRHACAECGKTYATSSNLSRHKQTHRSLDSQLARKCPTC 197
D. rerio      Scrt2    SESLSEGYTMDAFFISDGRSRRKGEVS--------------------------------EAAKADEVEKEVVG------VNNGGARHTCNECGKTYATSSNLSRHKQTHRSLDSKMARKCPTC 198
M. musculus   Scrt1    A-AVSEGYAADAFFITDGRSRRKAANANAAAAPSTASVAAPDSDAGGGGGPGTRGSGSGSASRGGTRVGAGTEARAGSGATGAGGRHACGECGKTYATSSNLSRHKQTHRSLDSQLARRCPTC 227
D. rerio      Scrt1a   T-AVSEGYTVDAFFITDGRSRRKAISS---------------------------------PR------------------TLQRHTCNECGKTYATSSNLSRHKQTHRSLDSKMAKKCPTC 166
D. rerio      Scrt1b   N-AVSEGYTVDAFFITDGRSRRKVISG---------------------------------SR------------------TLQRHTCNECGKTYATSSNLSRHKQTHRSLDSKMAKKCPTC 166

                       .*.******:***:***:*:***  :*.**********************.*:************************:  *::*.*:*.*****:**  *:**.:.          .     :
G. gallus     Scrt2    GKAYVSMPALAMHVLTHNLKHKCDVCGKAFSRPWLLQGHMRSHTGEKPFGCSHCGKAFADRSNLRAHMQTHSAFKHYKCKQCEKTFALKSYLNKHYESACFKG--------SEHSCAIGN-- 276
M. musculus   Scrt2    GKAYVSMPALAMHVLTHNLRHKCGVCGKAFSRPWLLQGHMRSHTGEKPFGCAHCGKAFADRSNLRAHMQTHSAFKHYRCRQCDKSFALKSYLHKHCEAACVKA--------AEPPPSAGPAS 311
D. rerio      Scrt2    DKVYVSMPALAMHILTHDLKHKCHVCSKAFSRPWLLQGHMRSHTGEKPFACAHCGKAFADRSNLRAHMQTHSAFKHYSCKRCNKTFALKSYLNKHYESACFRG-------SGDEDESGSEN- 312
M. musculus   Scrt1    GKVYVSMPAMAMHLLTHDLRHKCGVCGKAFSRPWLLQGHMRSHTGEKPFGCAHCGKAFADRSNLRAHMQTHSAFKHFQCKRCKKSFALKSYLNKHYESACFKGGASGPATPAPPQLSPVQA- 348
D. rerio      Scrt1a   GKVYVSMPAMAMHLLTHDLKHKCDICGKAFSRPWLLQGHMRSHTGEKPFGCAHCGKAFADRSNLRAHMQTHSAFKHYKCKRCNKTFALKSYLNKHYESACFKG--------AFAPLSPMEV- 279
D. rerio      Scrt1b   GKVYVSMPAMAMHLLTHDLKHKCDICGKAFSRPWLLQGHMRSHTGEKPFGCAHCGKAFADRSNLRAHMQTHSAFKHFKCKRCNKTFVLKSYLNKHYESACFKG--------AFSPHSSIEA- 279
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Fig. 1. Predicted protein sequence for chicken Scrt2 (*Gallus gallus*; AEW43643). Alignment with mouse Scrt1 (*Mus musculus*; NP_570963) and Scrt2 (NP_001153882) and zebrafish Scrt1a (*Danio rerio*; NP_001107073), Scrt1b (NP_001014369) and Scrt2 (NP_998802) and diagram showing positions of the previously described domains (Nieto, 2002) SNAG (aa 1–8), Scratch (aa 97–116) and 5 zinc fingers (Znf) (aa 127–150, aa 160–181, aa 185–207, aa 213–235 and 241–263) in the cScrt2 predicted amino acid sequence.

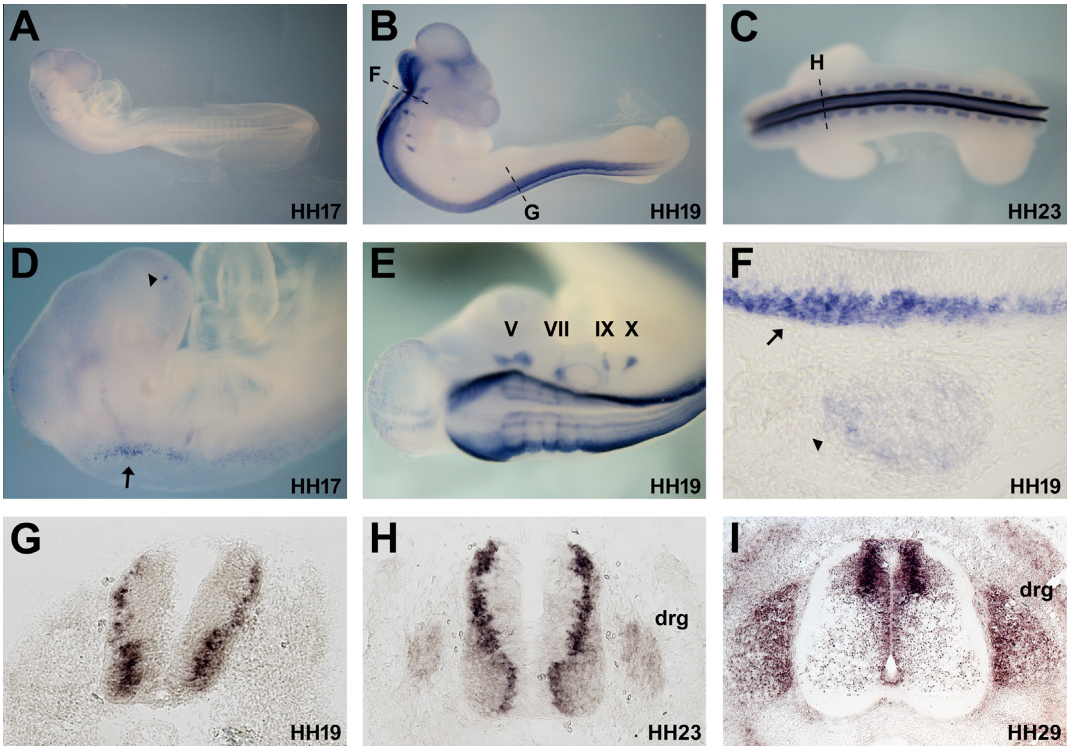

Fig. 2. Developmental expression of *cScrt2*. (A–E) Whole mount *in situ* hybridizations showing *cScrt2* expression in HH17 (A and D), HH19 (B and E) and HH23 (C). Dashed lines in (B) and (C) indicate sectioning plans and levels for (F–H). Arrow and arrowhead in (D) indicate positive cells in hindbrain and nasal placode, respectively. Roman numerals in (E) indicate cranial ganglia that express *cScrt2*. (F) Section of HH19 embryo subjected to whole mount *in situ* hybridization showing expression in the trigeminal ganglion (arrowhead); arrow indicates the hindbrain. (G–I) *In situ* hybridization in trunk spinal cord cross sections showing *cScrt2* expression in HH19 (G), HH23 (H) and HH29 (I). drg: dorsal root ganglia.

Thus, to better resolve Scratch's position in the neural differentiation transcriptional cascade in the vertebrate embryo, we cloned the full length coding sequence of the chicken *Scratch2* homolog (*cScrt2*) and characterized in detail its expression pattern relative to other known markers in the trunk neural tube.

1. Results and discussion

The full-length chicken orthologue of *Scrt2* was cloned by RT-PCR using primers designed against a partial clone previously filed in the NCBI database (XM_426994) and clones obtained with