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

Felipe Monteleone Vieceli<sup>a</sup>, Marcos Simões-Costa<sup>b</sup>, José Antonio Turri<sup>a</sup>, Tatiane Kanno<sup>a</sup>, Marianne Bronner<sup>b</sup>, Chao Yun Irene Yan<sup>a,\*</sup>

<sup>a</sup> Department of Cell and Developmental Biology, Universidade de São Paulo, São Paulo, SP, Brazil <sup>b</sup> 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.

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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).





<sup>\*</sup> 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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1			**********	. :		*: .	: .: * :		* .	* :*:		*: .:	
G.	gallus	Scrt2	MPRSFLVKKLKADAFPLAG	APAPPYAI	LEPPYALP	-GPAAGDGYLOH	CLAPA-GYNPD	KOG-LPPAPPDP	AY-APGOEEYS	DPESPC	STFSAR	YFNGE	95
м.	musculus	Scrt2	MPRSFLVKKIKADGFOCSG	VSAPTYHI	LETAYVLPGT	RGPPGDNGYVAH	CLPPS-GYDGE	KPG-LELAPAEP	AYPAAASEEYS	DPESPC	SSLSAR	YFRGE	99
Ь.	rerio	Scrt2	MPRSFLVKKIKLDDFSSSP	/SNHHHHHHNDRHMDDSFSI	SRSSLGVR	LCENGYIKD	YISSS-EYTEE	- KOAD-MKLNS-EL	LYSPVSSGGGEYC	OPDLEHPDSPC	SGLTARG	YFSSE :	113
м.	musculus	Scrt1	MPRSFLVKKVKLDTFSSA-	DLDSSYGH	ARSDLGVR	LODKGYLSD	YVGPASVYDGD	AEAALLKGPSPEP	MYAAAVRGELGPA	ASGSAPPPTPR	PELATAAGG	YINGD :	LOS
b.	rerio	Scrt1a	MPRSFLVKKVKLDDFSSS-	DLESSYGH	SRADISLR	FHEKAYISD	YMTPA-PYDGE:	SDSG-IKVPSPGP	IYDS-IHSDYG	APDSDOPDSPC	SEISSG	YINGD	97
b.	rerio	Scrt1b	MPRSFLVKKVKLDDFSSS-	ELESAYGH	SRTDLSFR	IHDKGYISD	YITPA-IYDGE	SDGG-TKVPSPGP	IYDS-NHSDYG	APDSDQPDSPC	SEITSG	YINGD	97
			SNAC										
			SNAG										
			::::.*: *****:***	***:					**:* *******	*******	****::*:	:****	
G.	gallus	Scrt2	A-AVTDSYSMDAFFITDGRS	SRRR			GE;	SQRRGSH	RHSCPECGKTYAT	SSNLSRHKQTH	RSLDSKMAR	KCPTC :	L64
М.	musculus	Scrt2	A-AVTDSYSMDAFFISDGRS	SRRRRAGAGGDAA		GAGDA	GGGGGGGGGGE	RAGRSGATAGGGH	RHACAECGKTYAT	SSNLSRHKQTH	RSLDSQLAR	KCPTC :	197
þ.	rerio	Scrt2	SESLSEGYTMDAFFISDGR	SRRKGEVS		EA	AKADEVEKEVV	GVNNGGA	RHTCNECGKTYAT	SSNLSRHKQTH	RSLDSKMAR	KCPTC :	198
М.	musculus	Scrt1	A-AVSEGYAADAFFITDGR	SRRKAANANAAAAPSTASV	APDSDAGGGG	GPGTRGSGSGSA	SRGGTRVGAGT	EARAGSGATGAGG	RHACGECGKTYAT	SSNLSRHKQTH	RSLDSQLAR	RCPTC :	227
þ.	rerio	Scrt1a	T-AVSEGYTVDAFFITDGRS	SRRKAISS			PR	TLQ	RHTCNECGKTYAT	SSNLSRHKQTH	RSLDSKMAK	KCPTC :	166
þ.	rerio	Scrt1b	N-AVSEGYTVDAFFITDGRS	SRRKVISG			SR	TLQ	RHTCNECGKTYAT	SSNLSRHKQTH	RSLDSKMAK	KCPTC :	166
									1				
1			Scratch						Zn	f 1	2	Znf 2	
1			Scratch						Zn	f1	2	Znf 2	
		-	Scratch	*** :*.********	*****	. * : * * * * * * * * *	* * * * * * * * * * * *	***: *::*.*:	Zn: * * * * * * * * *	f 1 **.:.	. :	Znf 2	
G.	gallus	Scrt2	Scratch	*** :*.********************************	***********	.*:********** GCSHCGKAFADR	*********** SNLRAHMQTHS	AFKHYKCKQCEKT	Zn *.****:** *: FALKSYLNKHYES	f 1 **.:. ACFKG	. : SEHSCAI	Znf 2	76
G. М.	gallus musculus	Scrt2 Scrt2	Scratch .*.*******:***:***:*** GKAYVSMPALAMHVLTHNLI GKAYVSMPALAMHVLTHNLI	:*** :*.************* CHKCDVCGKAFSRPWLLQGI CHKCGVCGKAFSRPWLLQGI	********** MRSHTGEKPF	.*:********* GCSHCGKAFADR GCAHCGKAFADR	********** SNLRAHMQTHS; SNLRAHMQTHS;	****: *::*.*: AFKHYKCKQCEKT AFKHYRCRQCDKS	Zn *.*****:** *: FALKSYLNKHYES FALKSYLHKHCEA	f 1 **.:. ACFKG ACVKA	SEHSCAI	Znf 2 GN 2 GPAS 33	76
G. M. D.	gallus musculus rerio	Scrt2 Scrt2 Scrt2	Scratch .*.****** GKAYVSMPALAMHVLTHNLI GKAYVSMPALAMHVLTHNLI DKVYVSMPALAMHILTHDLI	CHKCDVCGKAFSRPWLLQG CHKCGVCGKAFSRPWLLQG CHKCHVCSKAFSRPWLLQG	********** MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF	.*:********* GCSHCGKAFADR GCAHCGKAFADR ACAHCGKAFADR	*********** SNLRAHMQTHS SNLRAHMQTHS SNLRAHMQTHS	****: *::*.*: AFKHYKCKQCEKT AFKHYRCRQCDKS AFKHYSCKRCNKT	Zn *.*****:** *: FALKSYLNKHYES FALKSYLNKHYES FALKSYLNKHYES	f 1 **.:. ACFKG ACVKA ACFRG	SEHSCAI AEPPPSA -SGDEDESG	Znf 2 GN 2 GPAS 3 SEN- 3 VO2	76 L1 L2
G. M. D. M.	gallus musculus rerio musculus	Scrt2 Scrt2 Scrt2 Scrt1	Scratch .*.****** GKAYVSMPALAMHVLTHNLI GKAYVSMPALAMHVLTHNLI DKVYVSMPALAMHILTHDLI GKVYVSMPALAMHLLTHDLI GKVVYOMPANAMHULTHDLI	CHKCDVCGKAFSRPWLLQGI RHKCGVCGKAFSRPWLLQGI CHKCHVCSKAFSRPWLLQGI RHKCGVCGKAFSRPWLLQGI	********** MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF	.*:********* GCSHCGKAFADR GCAHCGKAFADR ACAHCGKAFADR GCAHCGKAFADR	*********** SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS;	****: *::*.*: AFKHYKCKQCEKT AFKHYRCRQCDKS AFKHYSCKRCNKT AFKHFQCKRCKKS AFKHFQCKRCCNCONT	Zn *.*****:** *: falksylnkhyes falksylnkhyes falksylnkhyes falksylnkhyes	f 1 **.:. ACFKG ACFKG-ASGPA ACFKGGASGPA	- SEHSCAI - AEPPPSA - SGDEDESG TPAPPQLSP	Znf 2 GN 2 GPAS 3 SEN- 3 VQA- 3 MEV 2	76 L1 L2 18
G. M. D. D.	gallus musculus rerio musculus rerio	Scrt2 Scrt2 Scrt2 Scrt1 Scrt1a	Scratch .*.**********************************	CHKCDVCGKAFSRPWLLQG RHKCGVCGKAFSRPWLLQG RHKCGVCGKAFSRPWLLQG RHKCGVCGKAFSRPWLLQG CHKCDICGKAFSRPWLLQG CHKCDICGKAFSRPWLLQG	********** MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF	.*:********* GCSHCGKAFADR GCAHCGKAFADR ACAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR	*********** SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS;	****: *::*.*: AFKHYKCKQCEKT AFKHYRCRQCDKS AFKHYSCKRCNKT AFKHFQCKRCKKS AFKHFYKCKRCNKT AFKHFYKCKRCNKT	Zn *.*****:** *: Falksylinkhyes Falksylinkhyes Falksylinkhyes Falksylinkhyes Falksylinkhyes	f 1 **.:. ACVKA ACVKA ACFRG ACFKGASGPA ACFKG	SEHSCAI AEPPPSA -SGDEDESG TPAPPQLSP AFAPLSP	Znf 2 GPAS 3: SEN- 3: VQA- 3- MEV- 2: TED- 2:	76 L1 L2 18 79
G. M. D. D. D.	gallus musculus rerio musculus rerio rerio	Scrt2 Scrt2 Scrt2 Scrt1 Scrt1a Scrt1b	Scratch .*.**********************************	CHKCDICGKAFSRPWLLQGI CHKCHVCSKAFSRPWLLQGI CHKCHVCSKAFSRPWLLQGI CHKCDICGKAFSRPWLLQGI CHKCDICGKAFSRPWLLQGI CHKCDICGKAFSRPWLLQGI	********** MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF <u>MRSH</u> TGEKPF	.*:********* GCSHCGKAFADR GCAHCGKAFADR ACAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR	*********** SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS;	AFKHYKCKQCEKT AFKHYRCRQCDKS AFKHYSCKRCNKT AFKHFQCKRCKKS AFKHYKCKRCNKT AFKH <u>FKCKRCNK</u> T	Zn *.*****:** *: FALKSYLNKHYES FALKSYLNKHYES FALKSYLNKHYES FALKSYLNKHYES	f 1 ACFKG ACVKA ACFRG ACFKGGASGPA ACFKG <u>A</u> CFKG	SEHSCAI -AEPPPSA -SGDEDESG TPAPPQLSP AFAPLSP AFSPHSS	Znf 2 GPAS 3: SEN- 3: VQA- 3- MEV- 2' IEA- 2'	76 L1 L2 18 79
G. M. D. D. D.	gallus musculus rerio musculus rerio rerio	Scrt2 Scrt2 Scrt2 Scrt1 Scrt1a Scrt1b	Scratch .*.****** GKAYVSMPALAMHVLTHNLI GKAYVSMPALAMHVLTHNLI DKVYVSMPALAMHILTHDLI GKVYVSMPAMAMHLLTHDLI GKVYVSMPAMAMHLLTHDLI Znf 2	KIKCDVCGKAPSRPWLLQG HKCGVCGKAPSRPWLLQG HKCGVCGKAPSRPWLLQG HKCHVCSKAPSRPWLLQG HKCD1CGKAPSRPWLLQG HKCD1CGKAPSRPWLLQG HKCD1CGKAPSRPWLLQG Znf 3	********** MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF <u>MRSH</u> TGEKPF	.*:********* GCSHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR <u>GCAHCGKAFADR</u> Znf	*********** SNLRAHMQTHS, SNLRAHMQTHS, SNLRAHMQTHS, SNLRAHMQTHS, SNLRAHMQTHS, SNLRAHMQTHS, SNLRAHMQTHS, 4	AFKHYKCKQCEKT AFKHYRCRQCDKS AFKHYSCKRCNKT AFKHYCKRCNKT AFKHYCKRCNKT AFKHYKCKRCNKT	Zn ******:** *: FALKSYLNKHYES FALKSYLNKHYES FALKSYLNKHYES FALKSYLNKHYES FVLKSYLNKHYES Znf 5	f 1 ACFKG ACVKA ACFRG ACFKGGASGPA ACFKG <u>A</u> CFKG	- SEHSCAI - AEPPPSA - SGDEDESG TPAPPQLSP - AFAPLSP - AFSPHSS	Znf 2 GPAS 3: SEN- 3: VQA- 3- MEV- 2: IEA- 2:	76 L1 L2 18 79 79
G. M. D. D. D.	gallus musculus rerio musculus rerio rerio	Scrt2 Scrt2 Scrt2 Scrt1 Scrt1a Scrt1b	Scratch KAYVSMPALAMHVLTHNLI GKAYVSMPALAMHVLTHNLI DKVYVSMPALAMHLLTHDLI GKVYVSMPAMAMHLLTHDLI GKVYVSMPAMAMHLLTHDLI GKVYVSMPAMAMHLLTHDLI Znf 2	CHRCDVCGRAFSRPWLLQG RHKCGVCGRAFSRPWLLQG HKCGVVCSRAFSRPWLLQG HKCDTCGRAFSRPWLLQG CHRCDTCGRAFSRPWLLQG CHRCDTCGRAFSRPWLLQG ZNf 3	********* MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF	.*:********* GCSHCGKAFADR GCAHCGKAFADR ACAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR Znf	************ SNLRAHMQTHSJ SNLRAHMQTHSJ SNLRAHMQTHSJ SNLRAHMQTHSJ SNLRAHMQTHSJ SNLRAHMQTHSJ	AFKHYKCKQCEKT AFKHYRCRQCDKS AFKHYSCKRCNKT AFKHFQCKRCKKS AFKHYRCKRCNKT AFKH <u>FKCKRCNKT</u>	Zn *.*****:** *: FALKSYLNKHYES FALKSYLNKHYES FALKSYLNKHYES FALKSYLNKHYES FVLKSYLNKHYES Znf 5	f 1 **.:. ACFKG ACVKA ACFKG ACFKG <u>A</u> CFKG	SEHSCAI AEPPPSA -SGDEDESG TPAPPQLSP AFAPLSP AFSPHSS	Znf 2 GPAS 3: SEN- 3: VQA- 3- MEV- 2' IEA- 2'	76 L1 L2 18 79
G. M. D. D. D.	gallus musculus rerio musculus rerio rerio	Scrt2 Scrt2 Scrt2 Scrt1 Scrt1a Scrt1b	Scratch KAYUSMPALAMHVLTHNLI GKAYUSMPALAMHVLTHNLI GKVYUSMPANAMHLLTHDLI GKVYUSMPANAMHLLTHDLI GKVYUSMPANAMHLLTHDLI GKVYUSMPANAMHLLTHDLI Znf 2	CHKCDVCGKAFSRPWLLQGH NHKCGVCGKAFSRPWLLQGI CHKCHVCSKAFSRPWLLQGI CHKCDTCGKAFSRPWLLQGI CHKCDTCGKAFSRPWLLQGI CHKCDTCGKAFSRPWLLQGI CHKCDTCGKAFSRPWLLQGI CHKCDTCGKAFSRPWLLQGI	********* MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF <u>MRSHT</u> GEKPF	GCSHCGKAFADR GCAHCGKAFADR ACAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR CAHCGKAFADR Znf	SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; A	AFKHYRCKQCBKT AFKHYRCRQCDKS AFKHYRCRQCDKS AFKHFQCKRCKKT AFKHFQCKRCKKS AFKHYRCKRCNKT	Zn *****:*** *: FALKSYLNKHYES FALKSYLNKHYES FALKSYLNKHYES FALKSYLNKHYES FVLKSYLNKHYES Znf 5	f 1 **.:. ACFKG ACVKA ACFKG ACFKG <u>A</u> CFKG <u>A</u> CFKG	- SEHSCAI - SEHSCAI - AEPPPSA - SGDEDESG TPAPPQLSP - AFAPLSP - AFSPHSS	Znf 2 GN 2 GPAS 3 SEN- 3 VQA- 3 MEV- 2 IEA- 2	76 L1 L2 18 79 79
G. M. D. D.	gallus musculus rerio musculus rerio rerio	Scrt2 Scrt2 Scrt2 Scrt1 Scrt1a Scrt1b	Scratch ,*,******;***;** GKAYUSMPALAMHVLTHNLI GKAYUSMPALAMHULTHDLI GKVYUSMPAMAMHLLTHDLI GKVYUSMPAMAMHLLTHDLI GKVYUSMPAMAMHLLTHDLI Znf 2	CHKCDVCGKAPSRPWLLQG UKCCVCGKAPSRPWLLQG UKCCVVCGKAPSRPWLLQG UKCCVCGKAPSRPWLLQG UKCD1CGKAPSRPWLLQG UKCD1CGKAPSRPWLLQG CHKCD1CGKAPSRPWLQG Znf 3	MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF	.*:********* GCSHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR Znf	SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; SNLRAHMQTHS; A	AFKHYKCKQCEKT AFKHYKCKQCEKT AFKHYSCKRCNKT AFKHFQCKRCKKS AFKHFKCKRCNKT AFKH <u>FKCKRCNKT</u>	Zn PALKSYLNKHYES PALKSYLNKHYES PALKSYLNKHYES PALKSYLNKHYES PVLKSYLNKHYES Znf 5	f 1 **.:. ACPKG ACVKA ACPKG ACFKGGASGPA ACPKG <u>A</u> CFKG	- SEHSCAI - AEPPPSA - SGDEDESG TPAPPQLSP - AFAPLSP - AFSPHSS	Cnf 2 GN 2 GPAS 3: SEN- 3: VQA- 3: MEV- 2: IEA- 2:	76 L1 L2 18 79
G. M. D. D.	gallus musculus rerio musculus rerio rerio	Scrt2 Scrt2 Scrt1 Scrt1a Scrt1b	Scratch .*.**********************************	CHKCDVCGKAPSRPWLLQG HKCGVCGKAPSRPWLLQG HKCGVCGKAPSRPWLLQG HKCGVCGKAPSRPWLLQG HKCDICGKAPSRPWLLQG (HKCDICGKAPSRPWLLQG CSCC ChCDICGKAPSRPWLLQG ChCDICGKAPSRPWLLQG CSCC CCCC CCCC CCCC CCCC CCCC CCCC CC	MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF MRSHTGEKPF	GCSHCGKAFADR GCAHCGKAFADR ACAHCGKAFADR GCAHCGKAFADR GCAHCGKAFADR CAHCGKAFADR Znf	SNLRAHMQTHSJ SNLRAHMQTHSJ SNLRAHMQTHSJ SNLRAHMQTHSJ SNLRAHMQTHSJ SNLRAHMQTHSJ A Znf 2	AFKHYKCKOCEKT PKHYRCKOCK AFKHYSCKRCNKT PFKHYCKRCNKT AFKHYKCKRCNKT AFKH <u>FKCKRCNKT</u>	Zn *	f 1 **:. ACPKG ACPKGGASGPA ACPKG <u>A</u> CPKG <u>A</u> CPKG	- SEHSCAI - AEPPPSA - SGDEDESG TPAPPQLSP - AFAPLSP - AFSPHSS	Znf 2 GPAS 3 SEN- 3 SEN- 3 VQA- 3 MEV- 2 IEA- 2	76 L1 L2 18 79 79
G. M. D. D.	gallus musculus rerio musculus rerio rerio	Scrt2 Scrt2 Scrt1 Scrt1a Scrt1b	Scratch KAYUSMPALAMHVLTHNLI GKAYUSMPALAMHVLTHNLI GKAYUSMPALAMHVLTHNLI GKVYVSMPAMAMHLLTHDLI GKVYVSMPAMAMHLLTHDLI GKVYVSMPAMAMHLLTHDLI Znf 2	CHKCDVCGKAFSRPWLLQGH HIKCGVCGKAFSRPWLLQG HKCHVCSKAFSRPWLLQG HKCDICGKAFSRPWLLQGH CHKCDICGKAFSRPWLCGH CHKCDICGKAFSRPWLLQGH CHKCDICGKAFSRPWLCGH CHKCDICGKAFSRPWCH CH	MRSHTGEKPP MRSHTGEKPP MRSHTGEKPP MRSHTGEKPP MRSHTGEKPP MRSHTGEKPP	stimulation destrogkapader deschedskapader deschedskapader deschedskapader deschedskapader deschedskapader Znf 2nf 1	SNLRAHMOTHS: SNLRAHMOTHS: SNLRAHMOTHS: SNLRAHMOTHS: SNLRAHMOTHS: SNLRAHMOTHS: 4 Znf 2	APKHYKCKQCEKT AFKHYRCRQCDKS AFKHYRCRQCDKS AFKHYRCKRCKKS AFKHYKCKRCNKT AFKH <u>FKCKRCNKT</u>	Zn * ***********************************	f 1 **.:. ACPRG ACPRG ACPRGG ACPRGGASGPA ACPKG ACPKG	- SEHSCAI - AEPPPSA - SGDEDESG TPAPPCLSP - AFAPLSP - AFSPHSS	Znf 2 GPAS 3: SEN- 3: VQA- 3: MEV- 2: IEA- 2:	76 L1 L2 18 79

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 *Scratch*2 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

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