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Mechanisms of Development 102 (2001) 223–226

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Gene expression pattern

Cloning and expression of a novel cysteine-rich secreted protein family member expressed in thyroid and pancreatic mesoderm within the chicken embryo

Devyn M. Smith[a], Lisa A. Collins-Racie[b], Valeria A. Marigo[a,1], Drucilla J. Roberts[a,2], Nicole M. Davis[a], Christine Hartmann[a], Ronen Schweitzer[a], Edward R. LaVallie[b], Laura Gamer[b], John McCoy[b,3], Clifford J. Tabin[a,*]

[a]*Department of Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA*
[b]*Genetics Institute, 87 Cambridgepark Drive, Cambridge, MA 02140, USA*

Received 3 August 2000; received in revised form 4 January 2001; accepted 14 January 2001

Abstract

We have isolated a new chicken gene that is a member of the cysteine-rich secreted protein family (CRISP). The CRISP family is composed of over 70 members that are found in many phyla of organisms, including: vertebrates, plants, fungi, yeast, and insects. Here we describe the cloning of a novel member of this family, *SugarCrisp*, and its expression pattern throughout chicken embryogenesis. We also describe its utility as a marker of thyroid and pancreatic mesoderm in the developing chicken embryo and its expression within the human and mouse in glandular tissue.

Keywords: Cysteine-rich secreted protein; Thyroid; Pancreas; Embryo

1. Results and discussion

The cysteine-rich secreted protein (CRISP) family is a large group of secreted proteins that function in some vertebrates, insects and plants as venoms and toxins (King, 1996; Schreiber et al., 1997). Several non-venomous family members have previously been described in the mouse and human, although the function of these proteins is unknown (Schwidetzky et al., 1995, 1997; Kaplan et al., 1999). These mammalian CRISPs are characterized by additional cysteine residues at their C-termini when compared to the venomous CRISPs (Schwidetzky et al., 1995, 1997). They are expressed within secretory glands of the adult animal, including the salivary and reproductive glands (Schwidetzky et al., 1995, 1997). We have cloned a novel member of the CRISP family. The protein encoded by this cDNA appears to be the homologue of a previously identified human protein termed P25TI, due to their 90% similarity at the amino acid level (Fig. 1; Yamakawa et al., 1998). We have termed this gene *SugarCrisp*, after the breakfast cereal.

The expression of *SugarCrisp* is first seen in the mesoderm of the emerging dorsal pancreatic bud at stage 17–18 (Hamburger and Hamilton, 1951) (Fig. 2A, white arrowhead). Section in situ hybridization confirms that this is mesodermal expression within the dorsal pancreatic bud (Fig. 2F–I, white arrowheads). Expression is also seen at this time in the thyroid anlagen as two circles of expression located between the emerging lung buds (Fig. 2B,F,H, red arrowheads). Expression persists throughout the dorsal pancreatic mesoderm through E6 as the pancreas continues to enlarge (Fig. 2C,D). After E6, the amount of mesoderm in the pancreas declines to a minimal level and little or no *SugarCrisp* expression is seen (data not shown). The expression in the mesoderm of the thyroid persists at least through E8 in the development of this organ (Fig. 2J). In addition to the expression in the pancreatic and thyroid mesoderm, there is a low level of expression of *SugarCrisp* within the emerging lung buds (Fig. 2A,G). We also detected SugarCrisp transcripts in regions of the developing gut. There is a

* Corresponding author. Tel.: +1-617-432-7618; fax: +1-617-432-7595.
E-mail address: tabin@rascal.med.harvard.edu (C.J. Tabin).
[1] Current Address: Telethon Institute of Genetics and Medicine, Via P. Castellino 111, 80131 Napoli, Italy.
[2] Current Address: Department of Pathology, Massachusetts General Hospital, Boston, MA 02114, USA.
[3] Current Address: Biogen, Inc. Fourteen Cambridge Center, Cambridge, MA 02142, USA.

0925-4773/01/$ - see front matter
PII: S0925-4773(01)00293-3

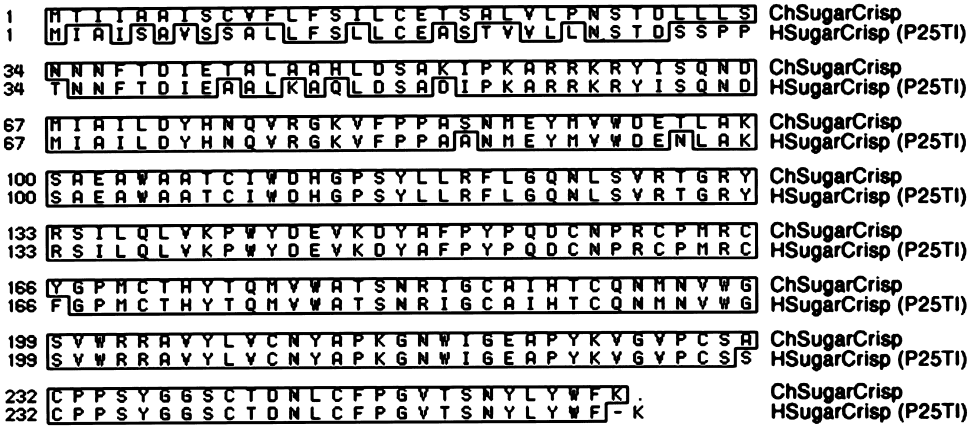

Fig. 1. Comparison of the protein sequences of chicken SugarCrisp and human SugarCrisp (P25TI).

stripe of expression at the junction between the proventriculus and the gizzard that does not encircle the entire circumference of the gut tube (Fig. 2D,E, green arrowheads). Faint expression is also detected in a domain just caudal to the pyloric sphincter (Fig. 2D, blue arrowhead), as well as in the connective tissue surrounding the small intestine (Fig. 2D,E).

Expression of *SugarCrisp* is also seen in the limb bud, without much staining in other embryonic regions at this stage (Fig. 3). This expression is seen as a patch of staining in the anterior as well as the posterior regions of the limb bud (Fig. 3, red arrowheads). The staining is much more prominent in the posterior limb bud than in the anterior limb bud (Fig. 3A–C). This expression is similar to that of the anterior and posterior necrotic zones in the limb bud (data not shown; Hinchliffe and Ede, 1973; Ferrari et al., 1998). Finally, at stage 21, staining is seen in the neck region (Fig. 3A, blue arrowhead). This staining becomes more intense by stage 26 and appears to be localized to the most anterior region of the heart (Fig. 3B,C, blue arrowheads).

We have also cloned the human (P25TI, Yamakawa et al.,

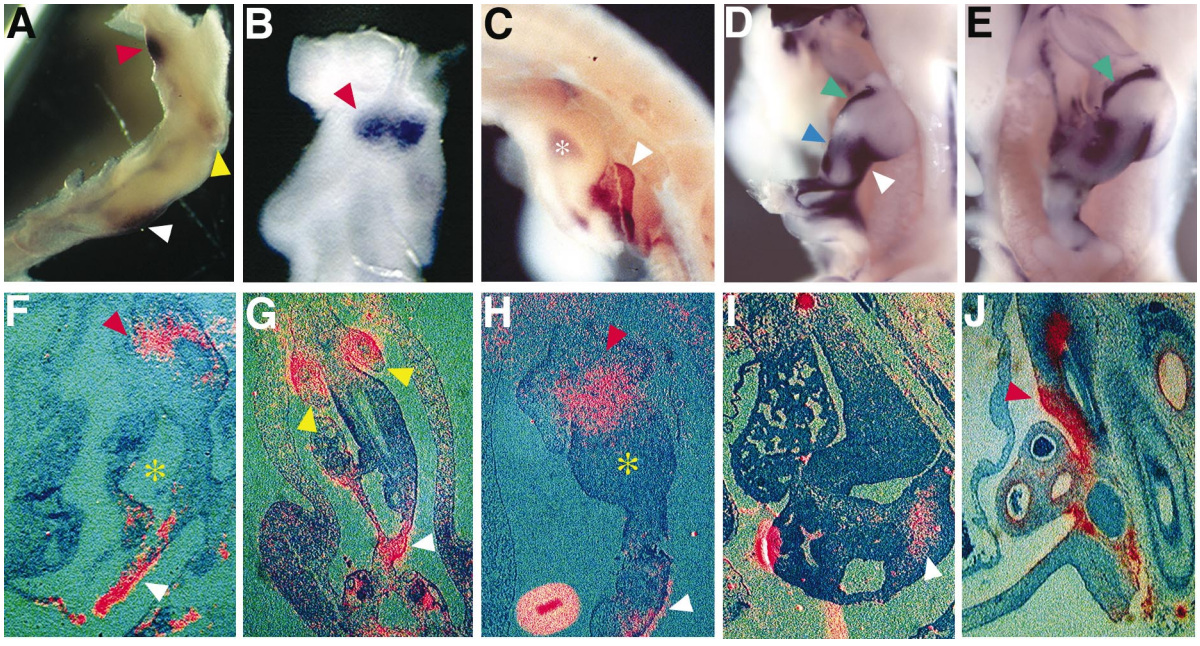

Fig. 2. Expression of chicken *SugarCrisp* in the thyroid, dorsal pancreas, gut and lung buds seen in whole mount and section in situ hybridization. (A,B) Stage 18 embryo. (C) E4 embryo. (D) E6 embryo, viewed from the left side. (E) E6 embryo, ventral view. (F) Longitudinal section through a stage 18 embryo. (G,H) Frontal sections through an E4 embryo. (I) Longitudinal section through an E5 embryo. (J) Longitudinal section through an E8 embryo. Red arrowheads point to staining in the thyroid; white arrowheads point to staining within the pancreas; yellow arrowheads indicate the position of the lung buds; green arrowheads indicate staining at the junction between the proventriculus and gizzard, the blue arrowhead points to staining caudal to the pyloric sphincter. Yellow asterisks denote the position of the gizzard in panels F and H. The white asterisk in panel C marks trapping of the riboprobe within the lumen of the gut and does not represent a true domain of SugarCrisp expression. Anterior is up in all panels.

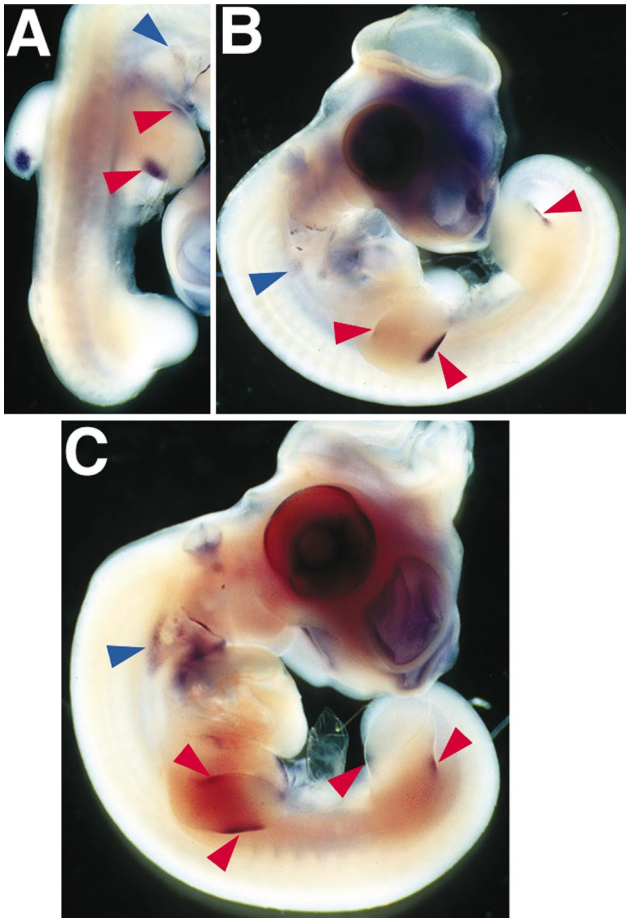

Fig. 3. Whole mount in situ hybridization of chicken *SugarCrisp* showing expression in the limb buds and neck region. (A) Stage 21 embryo. (B) Stage 23 embryo. (C) Stage 26 embryo. Red arrowheads point to limb staining, while blue arrowheads point to staining within the neck region. Staining within the head in panels B and C represents trapping of the riboprobe and not a real expression domain.

1998) and mouse homologues of *SugarCrisp*. While a thorough examination of expression in these organisms is beyond the scope of this study, we wanted to do a preliminary analysis of expression in adult tissues for comparison to the other vertebrate CRISP genes, which have only been reported in studies of adult mammals. To this end, we utilized commercially available multiple tissue blots to examine the expression of *SugarCrisp*. Like other known CRISP genes, *SugarCrisp* is expressed in a variety of adult glandular structures in humans and mice, including the prostate, mammary gland, salivary gland, and thyroid gland. *SugarCrisp* is also expressed in a number of organs where other CRISP genes have not been reported, including skeletal muscle, smooth muscle, heart, and the ovaries (data not shown).

2. Material and methods

The chicken *SugarCrisp* gene was cloned using a screen designed to isolate secreted molecules (Jacobs et al., 1997). We then used the chicken cDNA as a probe to screen human and mouse libraries for the mouse and human homologues of *SugarCrisp*. To generate a probe for in situ hybridization, we cloned the open reading frame for *SugarCrisp* into pBS. A riboprobe was generated by linearizing the construct with SacI and transcribing with T7 RNA polymerase. Whole mount and section in situ hybridization were performed as described (Riddle et al., 1993; Vortkamp et al., 1996). Human and mouse Master Blots were purchased from Clontech Laboratories, Inc. The mouse RNA masterblot was hybridized with a mouse *SugarCrisp* cDNA. The mouse *SugarCrisp* cDNA fragment was isolated using standard techniques. The labeled probe was denatured and hybridized overnight with the blot. The human cDNA was isolated and labeled in a similar manner and the labeled human *SugarCrisp* fragment was hybridized to the human blots. Blots were washed according to manufacturer specifications and exposed to film. Film was developed and analyzed using standard techniques.

Acknowledgements

This research was supported by the Genetics Institute and a grant to CJT from the NIH. We thank the members of the Cepko/Tabin group for their help and advice throughout this work. Mouse and chick SugarCrisp sequences have been deposited in GenBank, accession #AF329196 and #AF329195, respectively.

References

Ferrari, D., Lichtler, A.C., Pan, Z.Z., Dealy, C.N., Upholt, W.B., Kosher, R.A., 1998. Ectopic expression of Msx-2 in posterior limb bud mesoderm impairs limb morphogenesis while inducing BMP-4 expression, inhibiting cell proliferation and promoting apoptosis. Dev. Biol. 197, 12–24.

Hamburger, V., Hamilton, H.L., 1951. A series of normal stages in the development of the chick embryo. J. Morphol. 88, 49–82.

Hinchliffe, J.R., Ede, D.A., 1973. Cell death and the development of limb form and skeletal pattern in normal and wingless chick embryos. J. Embryol. Exp. Morphol. 30, 753–772.

Jacobs, K.A., Collins-Racie, L.A., Cobert, M., Duckett, M., Golden-Fleet, M., Kelleher, K., Kriz, R., LaVallie, E.R., Merberg, D., Spaulding, V., Stover, J., Williamson, M.J., McCoy, J.M., 1997. A genetic selection for isolating cDNAs encoding secreted proteins. Gene 198, 289–296.

Kaplan, F., Ledoux, P., Kassamali, F.Q., Gagnon, S., Post, M., Koehler, D., Deimling, J., Sweezey, N.B., 1999. A novel developmentally regulated gene in lung mesenchyme: homology to a tumor-derived trypsin inhibitor. Am. J. Physiol. 276, L1027–L1036.

King, T.P., 1996. Immunochemical studies of stinging insect venom allergens. Toxicon 34, 1455–1458.

Riddle, R.D., Johnson, R.L., Laufer, E., Tabin, C.J., 1993. Sonic hedgehog mediates the polarizing activity of the ZPA. Cell 75, 1401–1416.

Schreiber, M.C., Karlo, C.J., Kovalick, G.E., 1997. A novel cDNA from-*Drosophila* encoding a protein with similarity to mammalian cysteine-rich secretory proteins, wasp venom antigen 5, and plant group pathogenesis-related proteins. Gene 191, 135–141.

Schwidetzky, U., Haendler, B., Schleuning, W.D., 1995. Isolation and characterization of the androgen-dependent mouse cysteine-rich secretory protein-3 gene. Biochem. J. 309, 831–836.

Schwidetzky, U., Schleuning, W.D., Haendler, B., 1997. Isolation and characterization of the androgen-dependent mouse cysteine-rich secretory protein-1 gene. Biochem. J. 321, 325–332.

Vortkamp, A., Lee, K., Lanske, B., Segre, G.V., Kronenberg, H.M., Tabin, C.J., 1996. Regulation of rate of cartilage differentiation by Indian Hedgehog and PTH-related protein. Science 273, 613–622.

Yamakawa, T., Miyata, S., Ogawa, N., Koshikawa, N., Yasumitsu, H., Kanamori, T., Miyata, M., 1998. cDNA cloning of a novel trypsin inhibitor with similarity to pathogenesis-related proteins, and its frequent expression in human brain cancer cells. Biochim. Biophys. Acta 1395, 202–208.