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Mechanisms of Development 91 (2000) 387–391

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Gene expression pattern

Differential expression of glycogen synthase kinase 3 genes during zebrafish embryogenesis

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Received 19 July 1999; received in revised form 1 October 1999; accepted 4 November 1999

Abstract

Glycogen synthase kinase 3 (GSK-3) belongs to a highly conserved family of protein serine/threonine kinase whose members in high eukaryotes are involved in hormonal regulation, nuclear signaling, and cell fate determination. We have identified two zebrafish homologues related to mammalian GSK-3, ZGSK-3α and ZGSK-3β. ZGSK-3α was expressed uniformly from cleavage onward, and later was found in many but not all tissues, especially in the central nervous system, spinal cord, somites and pronephric ducts. ZGSK-3β was also transcribed maternally but the transcripts were not uniformly distributed during early cleavage stage. Most signals were concentrated in the inner part of the blastomeres. From midblastula stage onward, the ZGSK-3β transcripts remained confined to inner parts of the deep cell layer. During shield stage, both epiblast and hypoblast expressed the transcripts. After late gastrulation, the signals were detected ubiquitously. During segmentation, prominent ZGSK-3β signal was detected in head portion of the neural system. In the trunk, the expression was maintained in the neural tube and paraxial mesoderm and then became prominent in adaxial cells, followed by expression at the posterior region of somites. In pharyngula period ZGSK-3β transcripts were distributed in similar regions as those of ZGSK-3α, namely, neural tissues of the head portion, spinal cord and somites.

Keywords: Glycogen synthase kinase 3; Serine/threonine kinase; Reverse-transcription polymerase chain reaction; Central nervous system; Spinal cord; Somites; Pronephric ducts; Adaxial cells; Paraxial mesoderm; *MyoD*; Zebrafish

1. Results and discussion

Studies in *Drosophila*, *Xenopus* and mammalian cells have shown that GSK-3β plays a key role in the Wnt/wingless signaling pathway by negatively regulating β-catenin. In the absence of Wnt signals, a protein complex consisting of GSK-3β, axin, the adenomatous polyposis coli protein and protein phosphatase PP2A promotes the phosphorylation of β-catenin, thereby stimulating its degradation by the ubiquitin–proteosome system. While in the presence of Wnt signals, inhibition of GSK-3β activity allows the cytoplasmic accumulation of stabilized β-catenin, which then translocates to the nucleus, where it modulates target gene transcription and cell fate by interacting with members of the LEF1/TCF family of transcription factors (see reviews in Arias et al., 1999; Sokol, 1999).

We have identified two zebrafish homologues related to mammalian GSK-3, ZGSK-3α and ZGSK-3β. ZGSK-3α was highly conserved compared to other vertebrates, with about 90% identity to the kinase domain of mammalian GSK-3α. In addition to the highly conserved kinase domain, the N-terminal region of this protein contained a stretch of alanine-, glycine- and serine-rich regions which resembled the glycine- and serine-rich features of the N-terminal part of mammalian GSK-3α (Fig. 1). As to the ZGSK-3β, this form lacked the N-terminal feature of the α form, but shared 91–94% overall sequence identity with mammalian and *Xenopus* GSK-3β.

We first analyzed the temporal expression of ZGSK-3α and ZGSK-3β by RT-PCR. The results showed that ZGSK-3α gene was expressed maternally and the transcripts appeared relatively constant through 2 days after fertilization. However, relatively low level of ZGSK-3β transcripts was detected in blastulation and early gastrulation. Following late gastrulation, its expression levels increased and then remained constant through 2 days after fertilization (Fig. 2). This expression profile is in contrary to that in *Xenopus*, in

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0925-4773/00/$ - see front matter
PII: S0925-4773(99)00300-7

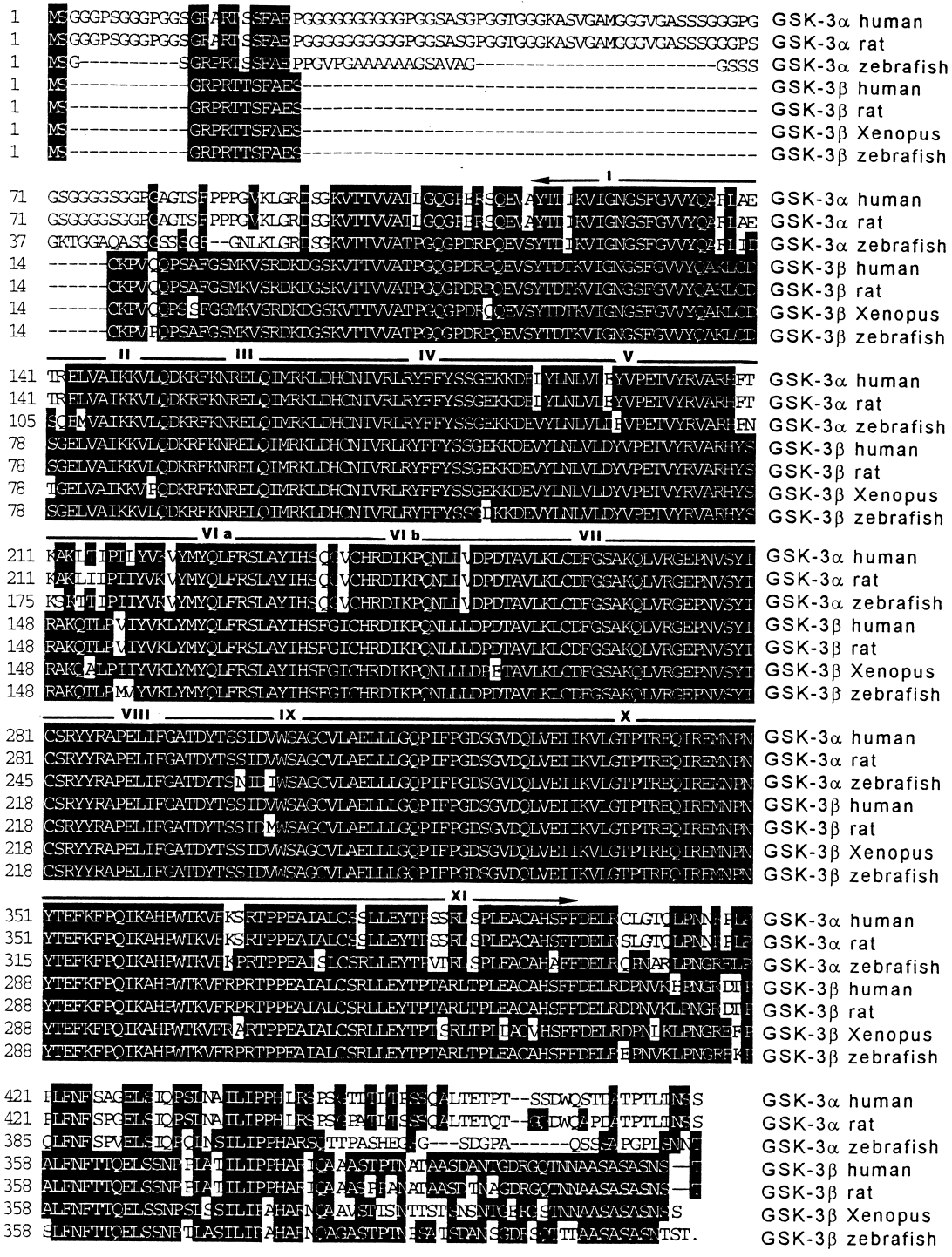

Fig. 1. Amino acid sequence alignment of the predicted zebrafish GSK-3α, GSK-3β, against the human GSK-3α (GenBank accession number D63424), human GSK-3β (Stambolic and Woodgett, 1994), rat GSK-3α, rat GSK-3β (Woodgett, 1990) and *Xenopus* GSK-3β (He et al., 1995). Consensus amino acid residues are shaded in black. The number at the left of each indicates the amino acid residue in each protein. Gaps indicated by hyphens are introduced for the optimal alignment. The 11 serine/threonine kinase subdomains (Hanks and Quinn, 1991) are marked with roman numerals.

which GSK-3β is expressed constantly throughout embryogenesis (Dominguez et al., 1995; Pierce and Kimelman, 1995).

The spatial expression pattern of GSK-3 genes was detected by whole-mount in situ hybridization. The results showed that ZGSK-3α was transcribed maternally and distributed uniformly except in yolk from cleavage stage through gastrulation (Fig. 3A,B). During segmentation, the signal remained uniform throughout the embryos except in the notochord (Fig. 3C,D). In the pharyngula period, the

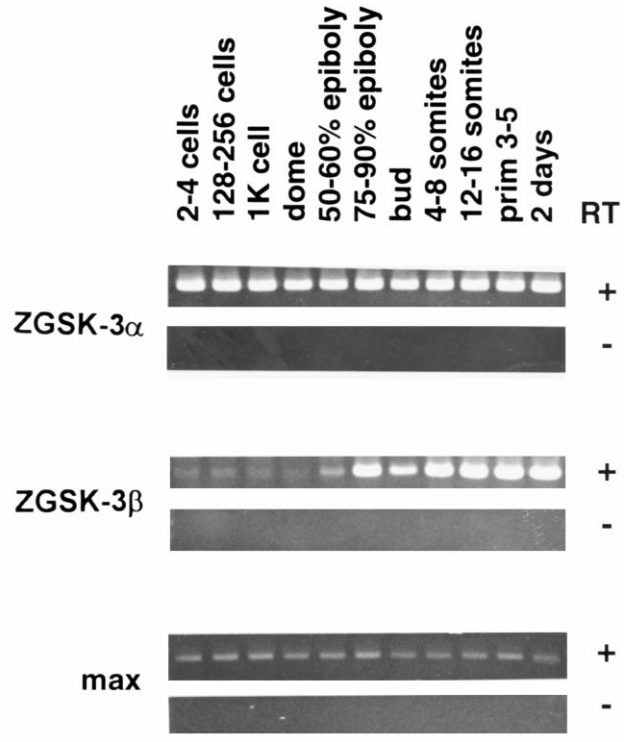

Fig. 2. RT-PCR analysis of the temporal expression pattern of ZGSK-3α, ZGSK-3β and *max* gene during zebrafish embryogenesis. The analysis was performed on cDNA synthesized from RNA isolated at selected developmental stages. Lane designated plus or minus symbol to the right of each refers to the presence or absence (control) of reverse transcription prior to PCR. Approximately equivalent amounts of cDNAs were being assayed as evident form the consistent level of the constitutively expressed *max* gene (Schreiber-Agus et al., 1993).

signal of ZGSK-3α mRNA varied among different tissues. More intense labeling was detected in the neural tissues at the head region, including the eye primordium (Fig. 3E). Moving caudally, ZGSK-3α was expressed in the lateral regions of the spinal cord, somites and pronephric ducts (Fig. 3F,G).

ZGSK-3β exhibited a spatial pattern of expression different from that of ZGSK-3α. ZGSK-3β was also transcribed maternally but the transcripts were not uniformly distributed in each blastomere during early cleavage stage. Most signals were confined to the inner part of the blastomeres and were negative in the interface between them (Fig. 4A,B). As cleavage proceeded, the signals became gradually localized in the deep cell layer (Fig. 4C,D). From the 1000-cell stage onward, the ZGSK-3β transcripts became confined to the inner parts of the deep cell layer, especially in the border regions near the vegetal pole, but were excluded in the yolk syncytial layer (Fig. 4E,F). During the shield stage, in addition to the deep cell layer, both epiblast and hypoblast in the germ ring expressed ZGSK-3β mRNA (Fig. 4G,H). After mid-gastrulation, the signals were located ubiquitously with apparently intensified expressions (Fig. 4I,J). This elevated expression was similarly observed by RT-PCR. The ZGSK-3β signal was detected in head portion of the neural system through segmentation period (data not shown). In the trunk, prominent ZGSK-3β expression was detected in adaxial cells at early segmentation (Fig. 4K). While weaker staining can be found in neural tube and unsegmented paraxial mesoderm throughout the segmentation stage (Fig. 4K–M). At the ten-somite stage, apparent ZGSK-3β transcripts began

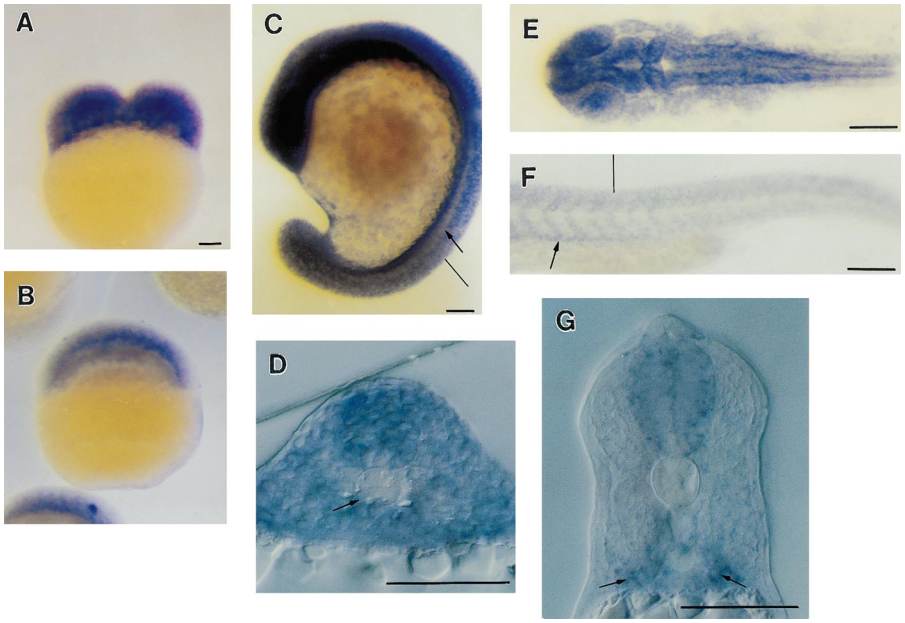

Fig. 3. Whole-mount in situ hybridization showing developmental expression pattern of ZGSK-3α. Lateral views (A–C,F). Dorsal view (E). D and G are cross sections of C and F, respectively, at the parts indicated by lines. (A) 2-cell stage. ZGSK-3α mRNA appeared maternally. (B) Gastrula stage. The transcripts were distributed uniformly except in yolk through the embryos. (C,D) Mid-segmentation stage, dorsal to the right and top in C and D, respectively. ZGSK-3α mRNA remained uniform through the embryos except in notochord (arrows in C,D). (E–G) Pharyngula period. The signals appeared in the neural tissues of the head region (E), the lateral regions of spinal cord (G), somites (F,G) and pronephric ducts (arrows in F,G). All scale bars: 100 μM.

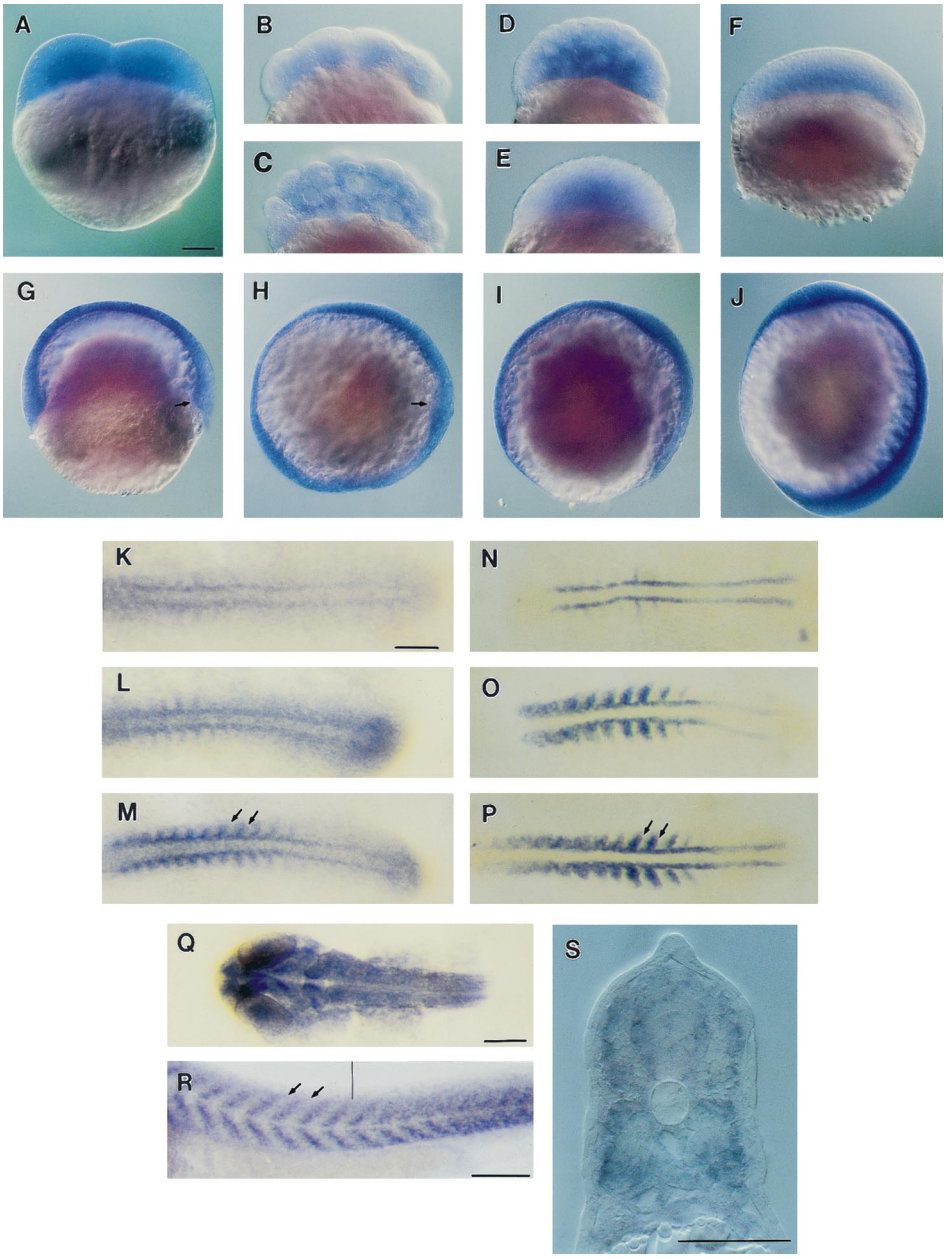

Fig. 4. Whole-mount in situ hybridization showing developmental expression pattern of ZGSK-3β. Zebrafish embryos were hybridized with ZGSK-3β (A–M,Q–S) or *MyoD* (N–P) probes. Lateral views (A–G,I–J,R). Animal pole view (H). Flat-mounted five-somite (K,N), ten-somite (L,O) and 14-somite stage (M,P) embryos. Dorsal view (Q). S is a cross section of R at the part of the line indicated. Animal pole is to the top in A–G and I. Dorsal is to the right in G–J. (A,B) two-cell and eight-cell stage. The distribution of the maternal ZGSK-3β mRNA was not uniform. Most signals were confined to inner parts of the blastomeres and were negative in the interface among them. (C) 64-cell stage. Some blastomeres became negative for ZGSK-3β mRNA. (D,E) 128-cell and 1000-cell stage. The signals became gradually localized in the inner part of the deep cell layer. (F) Thirty percent epiboly. After the epiboly formation, ZGSK-3β transcripts remained confined to the inner parts of the deep cell layer, especially in the border regions near the vegetal pole. Yolk syncytial layer is negative for the ZGSK-3β transcripts. (G,H) Shield stage. The arrows indicate shield. Both the epiblast and hypoblast in the germ ring expressed the ZGSK-3β transcripts. (I,J) Eighty percent epiboly and tail bud stage. After mid-gastrulation, the signals were located ubiquitously with apparently intensified expressions. (K) Five-somite stage. ZGSK-3β accumulated mainly in adaxial cells. A weak and diffuse labeling was observed in neural tube and paraxial mesoderm. (L) Ten-somite stage. The signal began to appear in somites. (M) Fourteen-somite stage. In somites, the expression was restricted to posterior portion. The arrows indicate intrasomitic furrows (also shown in P). (N) Six-somite stage. *MyoD* transcripts were expressed in adaxial cells and faint bands of cells in the somites. (O,P) Ten-somite and fourteen-somite stage. Prominent *MyoD* expression began to appear in the posterior portion of each somite. (Q–S) Pharyngula period. ZGSK-3β signal appeared in the neural tissues of the head region (Q), the lateral regions of spinal cord (S) and middle region of each somite (R). Arrows mark transversal myosepta. All scale bars: 100 μM.

to appear in the tail bud and cells that form discrete lateral extensions within the posterior region of the rostral somites (Fig. 4L). Similar ZGSK-3β expression occurred in subsequent somites pairs in a rostro-caudal progression as they

mature, while expression in the more caudal mesoderm remained confined to the unsegmented paraxial mesoderm (Fig. 4M). This mesodermal expression pattern was similar but distinct from that of *MyoD* in that the lateral extensions of *MyoD* RNA were expressed earlier and wider in size than those of ZGSK-3β (compare Fig.4K–M with N–P). In addition, *MyoD* transcript was not detected in neural tube and unsegmented paraxial mesoderm (Weinberg et al., 1996), while ZGSK-3β signal was expressed in these regions. In pharyngula periods ZGSK-3β transcripts were distributed in similar regions as those of ZGSK-3α, namely, neural tissues of the head portion, lateral regions of spinal cord and somites (Fig. 4Q–S). In the trunk, the gene was expressed at highest level in the middle of each chevron-shaped somite but was excluded in the region of horizontal myoseptum where the adaxial cell-derived muscle pioneer cells are localized (Devoto et al., 1996; Felsenfeld et al., 1991) (Fig. 4R).

In summary, the spatial expression patterns of ZGSK-3α and ZGSK-3β occurred first in different regions but converged to similar ones at later stages. Despite the differential expression pattern of zebrafish ZGSK-3β in contrast to the constitutive one in *Xenopus*, the spatial expression patterns of both in neural tissues and developing somites during segmentation was similar (Marcus et al., 1998).

2. Materials and methods

PCR with degenerate oligonucleotide primers designed to conserve motifs in the kinase domains of mammalian GSK-3 (Woodgett, 1990) using phages of a one-month-old zebrafish cDNA library (Clontech) as template led to a 333-bp DNA fragment. Based on the sequence information obtained from the cloned fragment, gene specific primers were designed and the RACE (rapid amplification of cDNA ends) method was exploited to isolate the full-length cDNAs. RT-PCR was performed using DNase-treated total RNA isolated from different stages of zebrafish embryos. Whole-mount in situ hybridization was performed as described by Schulte-Merker et al. (1992), except that hybridizations were carried out at 65°C. For sectioning, stained embryos were embedded in Epon and sectioned at 20 μm thickness (Westerfield, 1993).

Acknowledgements

The work is supported by grants (NSC 87-2312-B-001-005, NSC 88-2311-B-001-049) from the National Science Council of Republic of China. The authors thank Dr. Fu-Ming Pan for his assistance in DNA sequencing, Chiung-hsiang Cheng for his technical assistance in embryo embedding and sectioning. The cDNA sequences for ZGSK-3α and ZGSK-3β have been deposited to EMBL/GenBank with accession numbers AJ223501 and AJ223502, respectively.

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