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Cloning and characterization of a novel human Aurora C splicing variant

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Abstract

In the last 10 years, Aurora kinases have emerged as the key proteins regulating many events during cell mitosis. Despite the wealth of studies on human Aurora A and B, little is known about human Aurora C. Here we report a novel splicing variant of Aurora C, named as Aurora C-SV (Aurora C splicing variant), which encodes a 290-amino-acid protein. By RT-PCR analysis in various tissues, Aurora C-SV, like Aurora C, was found to be expressed at the highest level in human testis. The in vitro kinase assay showed that this Aurora C-SV phosphorylated MBP, and its T179A mutant lost the kinase activity. During cell mitosis, Aurora C-SV-EGFP associated with chromosomes in prophase and metaphase, and then transferred to the central spindle midzone and the cortex where the contract ring formed during the transition from anaphase to telophase. It then remained in the midbody during cytokinesis. Therefore, we speculated that Aurora C-SV might also contribute to the regulation of chromosome segregation and cytokinesis.

Keywords: Aurora C-SV; Kinase; Splicing variant; Phosphorylation; Mitosis

The proliferation of life relies on cell mitosis and meiosis. During these important processes, double-helix DNA is precisely replicated and then symmetrically segregated into two nascent daughter cells. To ensure genomic stability, accurate chromosome duplication and correct spindle dynamics are essential. These highly complex processes depend on precise regulation and control.

Reversible protein phosphorylation plays a key role in cell cycle control. The dynamic balance between phosphorylation and dephosphorylation of a series of protein kinases can either switch some mitotic events on or turn them off [1]. One class of the best-known kinase is cyclin-dependent kinase (Cdk) [2,3]. In addition, some other protein kinases are also known to be involved in many regulative systems, including the polo-like kinase (PLK) [4,5], the never in mitosis A family (NimA) [6], the Bub1 family [7,8], and the Aurora family.

The original Aurora allele, named as *Increase-in-ploidy1* (*Ipl1*), was identified in a screen for mutants that were defective in chromosome segregation in *Saccharomyces cerevisiae* [9]. Investigation in the past ten years has resulted in the discovery of many Aurora homologues in different model organisms. In *Homo sapiens*, there are three types of Aurora kinases (Aurora A, B, and C) which have a highly conserved catalytic C-terminal domain, and an N-terminal domain that is variant in size. Despite the obvious sequence and structural similarity, human Aurora A and B have distinct spatially and temporally regulated functions.

In somatic cells, Aurora A functions as an important regulator of centrosome maturation, assembly, and

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maintenance of a bipolar spindle and chromosome segregation [10]. Both the quantity and the kinase activity of Aurora A peak in mitosis [11]. The activity of human Aurora A requires the phosphorylation of its residue T288 in the activation loop [12]. Other molecules such as TPX2 (target protein for XKLP2) and p53 can regulate Aurora A by direct binding to distinct regions of Aurora A, in positive and negative ways, respectively [13–15]. Aurora B is responsible for chromosomal segregation and cytokinesis [16]. Its protein quantity and kinase activity are also at their peak in mitosis, but later than Aurora A [17]. In vivo, Aurora B together with Survivin and INCENP (inner centromere protein) form a chromosomal passenger complex, which plays an important role in regulating chromosomal segregation and cytokinesis. Once the complex is destroyed by deletion of any one of the three members, cells cannot complete normal mitosis, resulting in multinucleation [18].

Aurora C was first identified in a study of the general kinase expression profile in mouse sperm and eggs [19], and in a screen of human placental and testis cDNA library [20]. It is expressed at the highest level in the mammalian testis [19–21]. Its gene maps to chromosome 19q13.43 [21], a known region that shows deletion or rearrangement in some cancer cell genomes [22], and its protein product also shows overexpression in certain cancer cell lines [21] and primary colorectal cancers [23]. Aurora C protein level is low during S phase and peaks in G2/M phase, indicating its role in regulation of cell mitosis. In the earlier study Aurora C appeared at centrosomes in anaphase and persisted there until cytokinesis [21]. Recently, however, it has been revealed that subcellular localization of Aurora C was exactly the same as that of Aurora B, proving it to be a novel chromosomal passenger protein that cooperated with Aurora B to regulate mitotic chromosome dynamics in mammalian cells [24].

When Aurora C was amplified from human testis cDNA library, we accidentally obtained two Aurora C cDNAs. After sequencing, we found that one is similar to a cDNA sequence of Aurora C previously reported (GenBank Accession No. AF054621), but the other is a distinct splicing variant encoding an N-terminal shortened protein. We named this novel splicing variant Aurora C-SV. Through RT-PCR in 18 tissues, we found that Aurora C-SV, like Aurora C, was expressed the most in testis. The in vitro kinase assay showed that His-Aurora C-SV was able to phosphorylate MBP as Aurora A, B, and C. In order to characterize the subcellular localization of Aurora C and Aurora C-SV, we transiently transfected HeLa cells with these two kinases fused with EGFP. Aurora C and its novel splicing variant were also shown, during mitosis, to have a chromosomal localization during prophase and metaphase, to move to the spindle midzone when the sister chromatids start to separate, to subsequently relocate to the cortex of the contractile ring during telophase, and to remain in the midbody during cytokinesis. Judging from the consistent distribution during cell division, we suggest that this novel Aurora C-SV also contributes to chromosome segregation and cytokinesis, independent from its absent N-terminal regions.

Materials and methods

Cloning and sequencing of two Aurora C cDNAs. Two primers (FP: 5′-GGAAGTACCTCTCTGAGCGGTTG-3′ and RP: 5′-TCAGGAAGCCATCTGAGCACAG-3′) were designed to amplify Aurora C from the human testis cDNA library. PCR was performed using the PCR kit (Shenergy, China) for 34 cycles at 94 °C (60 s), 63 °C (60 s), and 72 °C (60 s). The products were separated by DNA electrophoresis in 1% (w/v) agarose gel, then cut out and purified with DNA purification kit (Sunbiotech, China). Each band was subcloned into pMD18-T vector (TaKaRa, Japan) and sequenced. The novel sequence data have been deposited with the EMBL/GenBank Data Libraries under Accession No. AY661554.

Tissue distribution of Aurora C-SV and Aurora C. Human MTC (multiple-tissue cDNA) panels (Clontech, USA) including bone marrow, stomach, bladder, lung, placenta, pancreas, heart, spleen, liver, thymus, testis, intestine, uterus, ovary, brain, skeleton muscle, and prostate served as templates to study the distribution of human Aurora C-SV and Aurora C mRNA with the same primers FP and RP as above. β-Macroglobin (β-MG) 5′ sense primer (5′ATGAGTATGCCTGCCGTGTGAAC3′) and 3′ antisense primer (5′TGTGGAGCAACCTGCTCAGATAC3′) were used to amplify a 290 bp amplicon of C-terminal of β-MG, which served as the internal control. Products were separated by DNA electrophoresis in 1% (w/v) agarose gel.

Plasmid construction and mutagenesis. For the bacterial expression of the fusion proteins, the Aurora A, Aurora C, and Aurora C-SV cDNAs were subcloned in-frame with His tag into the pET32a(+) (Amersham Biosciences, UK) and the Aurora B cDNA was inserted into pGEX4T-1 (Amersham Biosciences). To investigate subcellular localization, Aurora C and Aurora C-SV cDNAs were introduced into the pEGFP-N1 vector (Clontech). The enhanced green fluorescent protein (EGFP) sequence is located at the 3′ end of the kinase sequence. T179A mutant of Aurora C-SV was obtained by site-directed mutagenesis using the QuikChange site-directed mutagenesis kit (Stratagene, USA) according to manufacturer's instructions.

Preparation of recombinant proteins. The recombinant His- or glutathione *S*-transferase (GST)-tagged kinases were expressed in *Escherichia coli* strain BL21-CodonPlus(DE3)-RIL with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 25 °C after overnight or 3 h induction, respectively. Cells were collected and lysed in the buffer containing 50 mM NaH_2PO_4 (pH 8.0), 300 mM NaCl, 10 mM imidazole or the PBS buffer (pH 7.0), respectively, supplemented with 1% Triton X-100, 10 mM β-mercaptoethanol, 0.5 mM PMSF, and 1 mg/ml lysozyme. After incubation on ice for 30 min, the samples were centrifuged and the supernatants were purified using Ni–NTA His-bind Resins (Novagen, USA) or Glutathione–Sepharose 4B (Amersham Biosciences), respectively, as recommended by the manufacturers.

Western blotting. Recombinant proteins were resolved by 12% SDS–PAGE and then transferred to nitrocellulose membranes, and these were incubated with antibodies against the Aurora A (goat anti-aik1, Santa Cruz, USA), Aurora B (monoclonal anti-AIM1, BD, USA), and Aurora C (rabbit anti-Aurora C C-terminal, Abgent, USA), followed by horseradish peroxidase-conjugated rabbit anti-goat, anti-mouse, and goat anti-rabbit antibodies, respectively.

In vitro kinase assay. Reaction mixture (20 μl) containing enzymes, 3 μg of myelin basic protein (MBP) as the substrate, 5 μM ATP, and 10 μCi [γ-^{32}P]ATP in kinase buffer (500 mM Hepes, pH 7.4, 10 mM

$MgCl_2$, 10 mM $MnCl_2$, 1 mM EGTA, 1 mM DTT, and 5 mM β-glycerophosphate) was incubated at 30 °C for 30 min. Samples were separated by 12% SDS–PAGE and visualized using Coomassie brilliant blue staining. The gels were dried and subjected to autoradiography.

Cell culture and transfection. HeLa cells were cultivated in Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 10% fetal calf serum (Invitrogen, USA) at 37 °C in 5% CO_2-humidified atmosphere. 1.2×10^5 cells were seeded on coverslips in 35 mm dishes. After overnight growth cells were 80% confluent and transfected with 1 μg plasmid EGFP-N1-Aurora C or Aurora C- SV using Lipofectamine Reagent (Invitrogen) in the non-serum medium. After 5 h of incubation the medium was replaced with fresh complete medium, and cells were cultured for an additional 35 h before collection.

Fluorescence microscope. Cells grown on coverslips were fixed in pre-iced 3.7% formaldehyde/PBS (pH 7.0) for 10 min. After being washed with PBS for three times, cells were counterstained with 1 μg/μl of 4,6-diamidino-2-phenylindole (DAPI) at 37 °C for 20 min. Images were acquired using a LEICA DC 500 camera on a microscope equipped with LEICA DMRA2 fluorescent optics (LEICA, Germany).

Results

Molecular cloning of a human Aurora C splicing variant

Through PCR from the human testis cDNA library with the primers FP and RP, two different amplicons (1 and 2) of Aurora C were obtained at the same time (Fig. 1). After sequencing, we found that cDNA of amplicon 1 is 1170 bp and amplicon 2 is 978 bp. Online BLAST research (http://www.ncbi.nlm.nih.gov/blast) gave the result that the amplicon 1 sequence is similar to a mRNA sequence of Aurora C (GenBank Accession No. AF054621), but the former is just 54 bp more than the latter at the start of the 5′ terminal of exon 1. Since the rest of their mRNA sequences are identical, they have the same ORF. Sequence of Amplicon 2 (GenBank Accession No. AY661554) represents a novel splice variant of Aurora C (Aurora C-SV). The cDNA and amino acid sequences of Aurora C-SV are displayed in Fig. 2. Aurora C-SV cDNA encodes a putative 290 amino acid protein with a calculated molecular mass of 33.7 kDa.

Compared with Aurora C, the spicing model of Aurora C-SV only differs at one site, the 5′ splice site of intron 1 (Fig. 3). The 5′ splice site of intron 1 of Aurora C-SV lies just within the exon 1 of Aurora C. As a result,

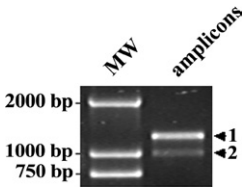

Fig. 1. Two amplicons of Aurora C amplified from human testis cDNA library. PCR amplification with the primers FP and RP using the human testis cDNA library as the template. Two different amplicons (1 and 2) of Aurora C were obtained. Amplicon 1 is 1170 bp and Amplicon 2 is 978 bp.

```
1   ggaagtacctctctgagcggttggtgccgggtataaaagaaggccgcgcagccacggctg
61  ctcacgacgccgcggatcccgaagcctgtgtagcactgagacatcatggctacagcaaac
                                                  M  A  T  A  N
121 caaacagcccagcagcccagcagcccagccatgcggcgcctcacagtcgatgactttgaa
    Q  T  A  Q  Q  P  S  S  P  A  M  R  R  L  T  V  D  D  F  E
181 atcgggcgtcccctgggcaaggggaaatttgggaatgtgtacctggctcggctcaaggaa
    I  G  R  P  L  G  K  G  K  F  G  N  V  Y  L  A  R  L  K  E
241 agccatttcattgtggccctgaaggttctcttcaagtcgcagatagagaaggaaggactg
    S  H  F  I  V  A  L  K  V  L  F  K  S  Q  I  E  K  E  G  L
301 gagcaccagctgcgccgggaaattgagatccaggctcatctacaacaccccaatatcctg
    E  H  Q  L  R  R  E  I  E  I  Q  A  H  L  Q  H  P  N  I  L
361 cgcctgtataactatttccatgatgcacgccgggtgtacctgattctggaatatgctcca
    R  L  Y  N  Y  F  H  D  A  R  R  V  Y  L  I  L  E  Y  A  P
421 aggggtgagctctacaaggagctgcagaaaagcgagaaattagatgaacagcgcacagcc
    R  G  E  L  Y  K  E  L  Q  K  S  E  K  L  D  E  Q  R  T  A
481 acgataatagaggagttggcagatgccctgacctactgccatgacaagaaagtgattcac
    T  I  I  E  E  L  A  D  A  L  T  Y  C  H  D  K  K  V  I  H
541 agagatattaagccagagaacctgctgctggggttcaggggtgaggtgaagattgcagat
    R  D  I  K  P  E  N  L  L  L  G  F  R  G  E  V  K  I  A  D
601 tttggctggtctgtgcacacccctccctgaggaggaagacaatgtgtgggacactggac
    F  G  W  S  V  H  T  P  S  L  R  R  K  T  M  C  G  T  L  D
661 tacttgccgccagaaatgattgaggggagaacatatgatgaaaaggtggatttgtggtgc
    Y  L  P  P  E  M  I  E  G  R  T  Y  D  E  K  V  D  L  W  C
721 attggagtgctctgctatgagctgctggtgggatatccacccctttgagagcgcctcccac
    I  G  V  L  C  Y  E  L  L  V  G  Y  P  P  F  E  S  A  S  H
781 agtgagacttacagacgcatcctcaaggtagatgtgaggtttccactatcaatgcctctg
    S  E  T  Y  R  R  I  L  K  V  D  V  R  F  P  L  S  M  P  L
841 ggggcccgggacttgatttccaggcttctcagataccagcccttggagagactgcccctg
    G  A  R  D  L  I  S  R  L  L  R  Y  Q  P  L  E  R  L  P  L
901 gcccagatcctgaagcacccctgggttcaggcccactcccgaagggtgctgcctcctgtt
    A  Q  I  L  K  H  P  W  V  Q  A  H  S  R  R  V  L  P  P  C
961 gctcagatggcttcctga
    A  Q  M  A  S  *
```

Fig. 2. Nucleotide and deduced amino acid sequences of human Aurora C-SV cDNA. This novel cDNA encodes a 290-amino acid protein. PCR primers are underlined. Nucleotide numbers are shown at the left of each line. These sequences are available from GenBank Database under GenBank Accession No. AY661554.

the exon 1 of Aurora C-SV is, in its 3′ terminal, 192 nt shorter than that of Aurora C, and then 19 amino acids are absent in the N-terminal of Aurora C-SV. The rest of the 6 exons of Aurora C-SV are exactly the same as those of Aurora C, and except for the absent 19 amino acids, the amino acid sequence of Aurora C-SV is consistent with that of Aurora C.

Analysis of conserved subdomains of Aurora C-SV with other human Aurora family members

Alignment of amino acid sequences among Aurora A, Aurora B, Aurora C, and Aurora C-SV using BioEdit sequence alignment software showed that the novel Aurora C-SV contains all the residues critical for protein kinase activity [25] and 11 known conserved subdomains in the catalytic kinase domain [26] (Fig. 4). By the analysis of NCBI-BLASTp (http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html), we found that the C-terminal catalytic domain of Aurora C-SV, which is equal to that of Aurora C, shares 84% identities and 91% positives with Aurora B, and 71% identities and 84% positives with Aurora A. In the activation loop, the threonine 288 of Aurora A [12], threonine 232 of

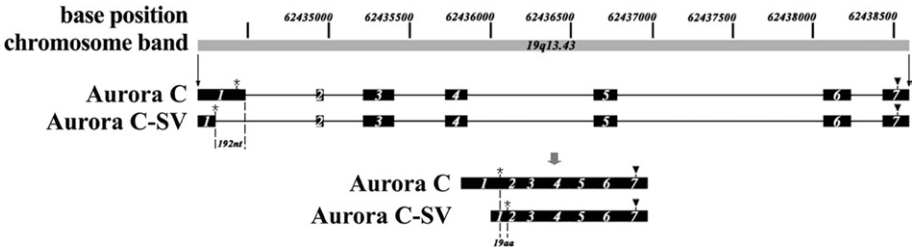

Fig. 3. Schematic illustration of the alternative splicing of Aurora C pre-mRNA. Exons 1–7 are shown as filled boxes. Two different splicing variants (Aurora C and Aurora C-SV) identified in this work are shown. The exon 1 of Aurora C-SV is 192 nt shorter than that of Aurora C. Start codons are indicated by asterisks and stop codons by triangles. Two mature mRNAs differ in the 5′ terminal and the protein product of Aurora C-SV is 19 aa less than Aurora C.

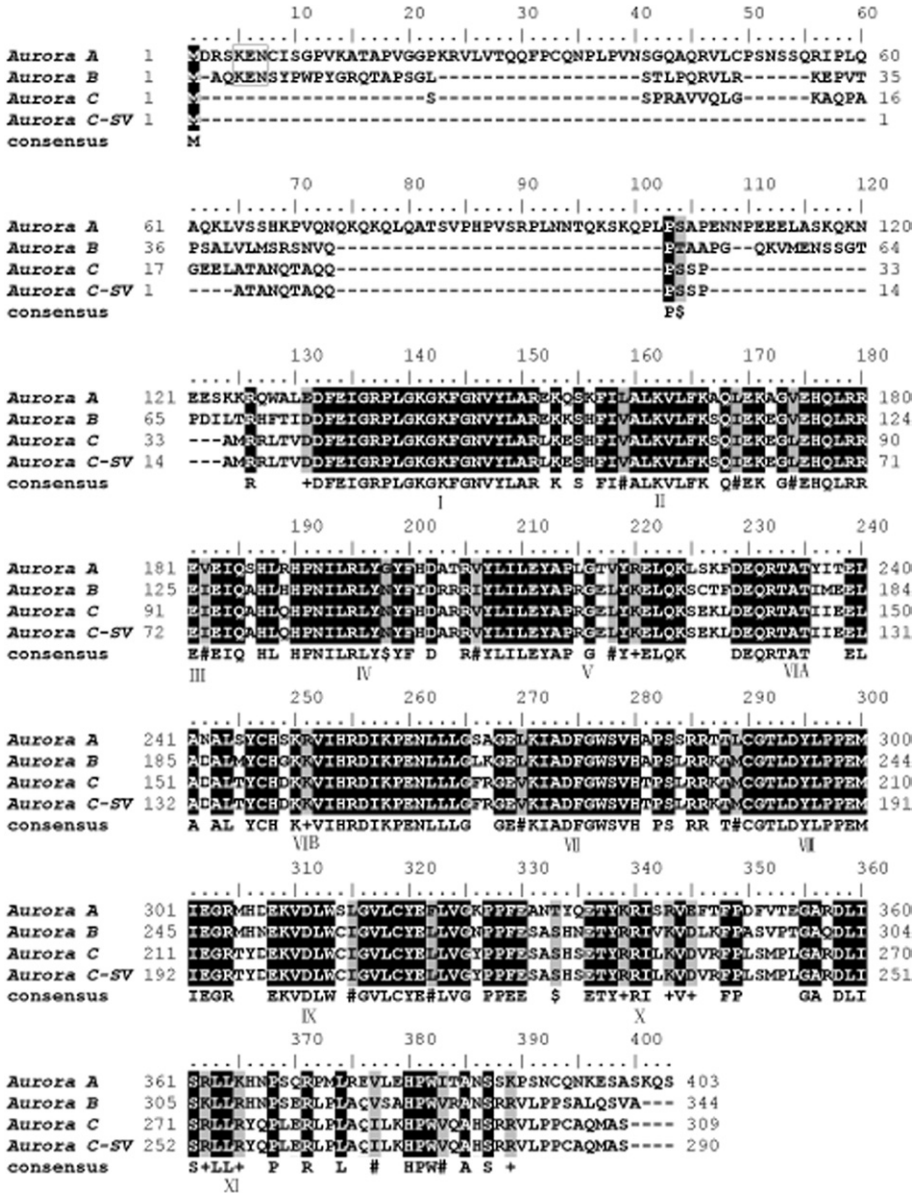

Fig. 4. Amino acid sequence alignment of human Aurora A, B, C, and C-SV. The sequences were aligned by Clustal X [27] with manual editing. The consensus sequence was calculated using BioEdit tool [28]. The identical sequences are boxed in black and the similar are in gray. The lower-case letters: invariant residues. #, conserved nonpolar residues; $, conserved polar and noncharged residues; +, conserved polar and charged residues. Eleven conserved subdomains of human Aurora kinase family are indicated by roman numbers. Amino acid numbers are shown at the left and right of each line. The KEN motif, absent in both Aurora C and C-SV, is boxed.

Aurora B [29], threonine 198 of Aurora C [24], and threonine 179 of Aurora C-SV (see the result '*Kinase activity of Aurora C-SV and T179A mutant in vitro*') are all phosphorylated residues that contribute to the kinase activity. However, both Aurora C-SV and Aurora C have a truncated N-terminal and lack a conserved motif 'KEN' that is present in Aurora A and B (Fig. 4). In fact, their N-terminal domains share low identity in length and sequence with other Aurora family members.

Expression pattern of Aurora C-SV

High expression levels of Aurora C in testis have been identified in human and mouse by Northern blot analyses. Here, by RT-PCR, we further observed the distribution of Aurora C-SV mRNA in commercially prepared cDNAs of 18 human tissues. The primers FP and RP that we used in cloning Aurora C-SV were reused for the PCR amplification. Two amplicons (Fig. 5, the upper band: Aurora C, the lower band: Aurora C-SV) were strongly visualized again in the amplification product from human testis, and the amount of Aurora C-SV cDNA was much less than that of Aurora C. Except for lung and placenta, which showed very faint positive bands, neither the full-length mRNA of Aurora C-SV nor that of Aurora C was detected in the other 15 tissues. In all, Aurora C-SV expresses at the highest level in human testis, consistent with Aurora C.

Kinase activities of Aurora C-SV and its T179A mutant in vitro

To investigate whether Aurora C-SV indeed exhibits kinase activity and determine the extent of this activity in comparison with those of other four members of human Aurora kinases, the fusion proteins of His-Aurora C-SV and other members of His-Aurora A, GST-Aurora B, and His-Aurora C were obtained from bacteria. The in vitro kinase activities of these four proteins were determined using MBP (myelin basic protein) as the substrate. Fig. 6A shows that Aurora C-SV also has the ability to phosphorylate MBP, which proves that Aurora C-SV, which possesses complete kinase subdomains, has the conserved kinase activity. Nevertheless, compared with His-Aurora A, GST-Aurora B, and His-Aurora C, the band intensity of MBP phosphorylated by His-Aurora C-SV was the weakest.

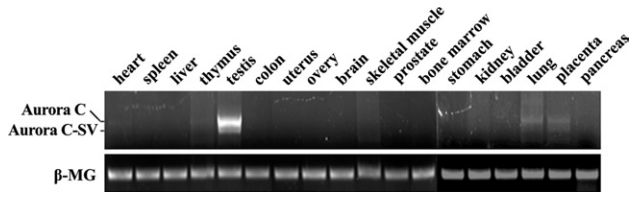

Fig. 5. Expression patterns of human Aurora C-SV and Aurora C. Both Aurora C-SV and Aurora C were found to be expressed at the highest level in human testis. Other two tissues, lung and placenta, seemed to express Aurora C and Aurora C-SV at a trace level.

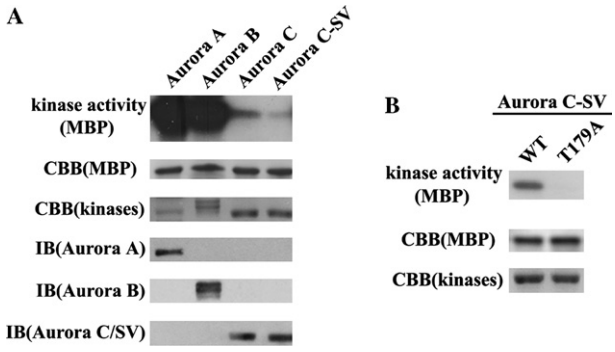

Fig. 6. In vitro kinase assay of His-Aurora C-SV and its T179A mutant. (A) The kinase activity of all human Aurora homologues. Kinase activity was measured in in vitro kinase assays with MBP as an exogenous substrate. MBP (3 μg) and fusion proteins (about 0.1 μg): His-Aurora A, GST-Aurora B, His-Aurora C, and His-Aurora C-SV were visualized by Coomassie blue staining (CBB). Four kinases were determined by Western blotting using corresponding antibodies. (B) The kinase activity of His-Aurora C-SV T179A mutant. MBP (3 μg) and fusion proteins (about 0.5 μg): His-Aurora C-SV and its T179A mutant were performed in the same condition described above.

Next, we induced a point mutation at Thr179 (corresponding to Thr198 in Aurora C) into Aurora C-SV. Thr 179 is in the activation loop of the catalytic domain between subdomains VII and VIII. Its equivalent residues in many other kinases including Aurora A, B, C, cAMP dependent kinase, and Cdc2 [30] were frequently reported to be critical to the kinase activity. Here we found that His-Aurora C-SV T179A mutant had a dramatically decreased kinase activity (Fig. 6B) in the kinase assay.

Subcellular localization of Aurora C and Aurora C-SV in HeLa cells

To obtain the accurate localization of Aurora C and Aurora C-SV in the process of mitosis, we transiently expressed Aurora C-EGFP and Aurora C-SV-EGFP in HeLa cells, respectively. Consistent with recent discovery [24], we also found that Aurora C, as well as Aurora C-SV, did not appear at the centrosomes, but distributed in the condensed chromosomes during prophase to metaphase. After entering anaphase, they dissociated from separated chromosomes and redistributed to midzone microtubules, and finally they remained in the midbody during cytokinesis (Fig. 7). All these results are exactly consistent with the subcellular localization of Aurora B [29], which is known as a significant chromosome passenger protein in mammalian cell mitosis, suggesting that the novel Aurora C-SV protein is also involved in mitotic events such as chromosome segregation and cytokinesis.

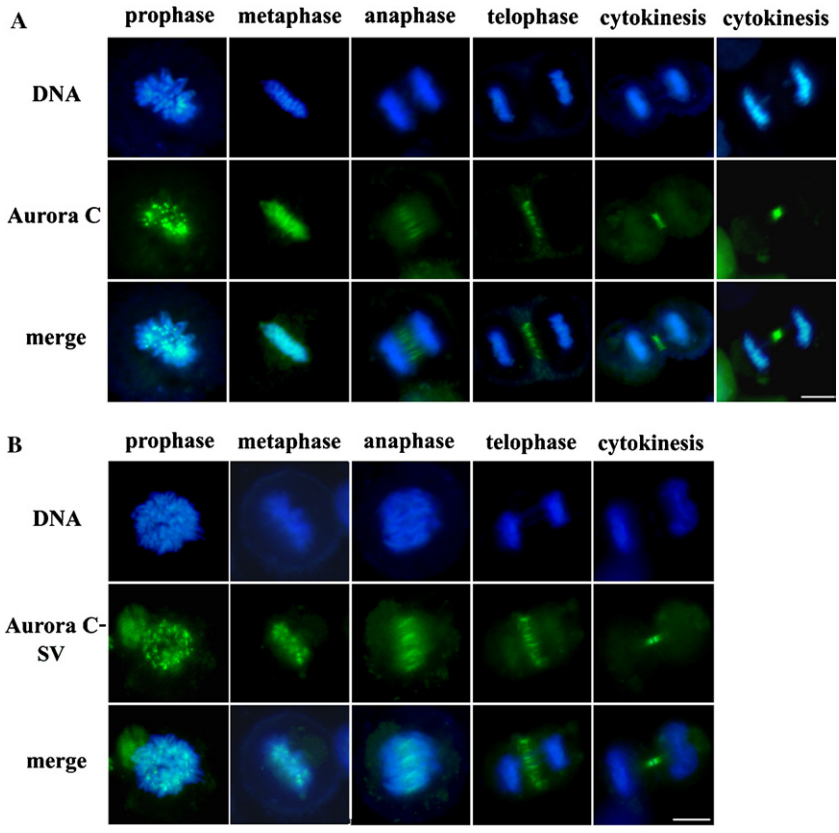

Fig. 7. Subcellular localization of Aurora C and Aurora C-SV. (A) Dynamic distribution of Aurora C-EGFP in HeLa cells. Aurora C (green) distributed in condensed chromosomes in prophase and then metaphase. During anaphase, Aurora C dissociated from chromosomes and redistributed to midzone microtubules. It subsequently relocated to the cortex of the contractile ring in telophase. During cytokinesis, just liked a disc, it telescoped between the two daughter cells and then became smaller and smaller until it looked like two small, proximate dots. Cells were counterstained with DAPI (blue). The scale bar represents 10 μm. (B) Dynamic distribution of Aurora C-SV-EGFP in HeLa cells. The subcellular distribution of Aurora C-SV (green) was consistent with Aurora C. Cells were counterstained with DAPI (blue). The scale bar represents 10 μm.

Discussion

In the past few years, initial reports on the sequences of Aurora C differed from each other, especially within the N-terminal [31]. Two groups that had investigated on AIE2/AIK3 [19,21] reported the similar mRNA sequences of Aurora C (GenBank Accession Nos. AF054621 and AB017332) whose open reading frame encodes a 309-amino-acid protein kinase with a 39-residue N-terminal region. By comparison, another group [20] identified a longer mRNA of 1327 bp (GenBank Accession No. AF059681) encoding a 275-amino-acid protein with a shorter N-terminal. These mRNA splicing models are mainly different in the 5′ terminal regions (Figs. 8A and B). In order to get the Aurora C cDNA that encodes the 275-amino-acid kinase from human testis cDNA library, we designed a 23 bp-length oligonucleotide within the exon 1 region of AF059681 but not in that of AF054621 and AB017332 as the forward primer, and another that locates around the stop codon as the reverse primer. Inconsistent with our expectation, however, we acquired two different sequences (Figs. 8C and D). One sequence (amplicon 1 in this work) basically coincides with those of AF054621 and AB017332, but has an extended exon 1 at 5′ terminal. Another sequence (amplicon 2 in this work) we named as Aurora C-SV is a novel sequence which has a longer intron 1, because the new 5′ splice site of intron 1 is 192 bp upstream of the old one. Therefore, the novel splicing loses the original start codon, and the new ATG is at the junction of exon 1 and 2 since there is a stop codon TGA 9 bp upstream. The novel Aurora C-SV has an open reading frame of 873 bp, encoding a putative 290-amino-acid protein.

Studies on the evolution of alternative splicing tell us there are five major forms of alternative splicing that have been found to date. Exon skipping, also known as cassette exon, accounts for 38% of the alternative splicing events conserved between human and mouse genomes. Alternative 5′ and 3′ splice sites account for 18% and 8% of the conserved events, respectively. Intron retention is responsible for 3% and other more complex events account for the remaining 33% [32]. Obviously, the splicing model of Aurora C-SV belongs to the alternative 5′ splice site. Based on the opinion in the review by Gil, some of the mutations that

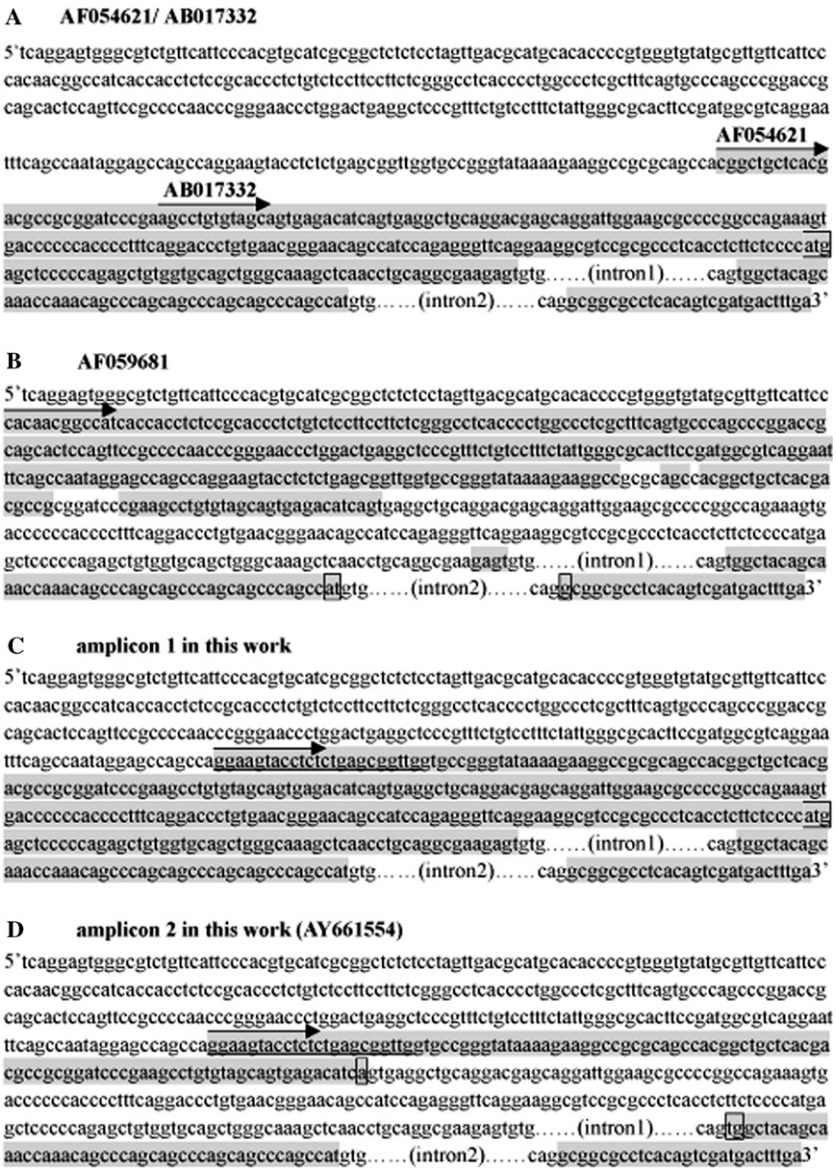

Fig. 8. Different splice sites in the 5′ terminal of Aurora C gene. The DNA sequences come from human genome and range from chr19: 62418099 to chr19: 62419448. Exons are shaded and the start codons are boxed. Each arrow represents the start of exon 1 of each sequence, which is according to the GenBank accession number and the result of this work. The primer that was used is underlined. (A,B) 5′ terminal splice sites of the published sequence AF054621/AB017332 and AF059681; (C,D) 5′ terminal splice sites of the sequences we got from human testis cDNA library with FP and RF described in the Materials and methods.

accumulated in a new 5′ splice site lead to activation of this site and generation of a new transcript. If the newly created splicing variant could acquire a new function that presents an advantage to the organism or, at least, it is not deleterious, it might be retained. This is a way of enriching the transcriptome and enhancing the coding capacity and regulatory versatility of the genome with new isoforms.

In general, there is only a slight difference between Aurora-SV and Aurora C in the sequence of non-catalytic domain. Like Aurora C, Aurora-SV is expressed at the highest level in human testis, possesses the serine/threonine kinase activity, and dynamically translocates from chromosomes during prophase and metaphase to midbody during cytokinesis in tumor cell division. Although Aurora C-SV only occupies a minor proportion of total Aurora C transcript, we still consider that it may participate in sperm development and can regulate cancer cell mitosis, probably as an isoform of Aurora C.

Given the catalytic domain is exactly the same between Aurora C-SV and Aurora C, Aurora C-SV was expected to show the kinase activity. The in vitro kinase assay actually demonstrated this. However,

compared with three other kinases in the same family, it has a significantly decreased activity. The N-terminal was reported to affect Aurora A in the APC/C mediated degradation pathway which was not found to be the case in Aurora B because it loses the A-box within the N-terminal [33]. Recently, we found that the N-terminal region affected the kinase activity of Aurora A in vivo (unpublished data). From human Aurora A to Aurora C-SV, the N-terminal becomes shorter and shorter, and the kinase activity less and less. Whether the shortened N-terminal is responsible for the decrease of kinase activity requires further investigation.

Three human Aurora homologues have typical subcellular localizations and individual function in different stages of cell mitosis. Although these three kinases are highly conserved in the catalytic domain, the N-terminal region varies a lot in both sequence and length. As a result, the question was raised as to whether or not Aurora's N-terminal sequence has a relationship with its localization. In *Xenopus* egg extracts, the N-terminal domain of pEg2 localized to the centrosomes in a microtubule-dependent manner and overexpression of the N-terminal inhibited bipolar spindle assembly [34]. Interestingly, the single catalytic domain could also localize to the centrosome but in a microtubule-independent manner. One speculation is that pEg2 kinase would localize to the centrosome, with its N-terminal domain directly binding to microtubules and the catalytic domain directly interacting with the substrates. If the N-terminal were absent, pEg2 would be at the original position through directly binding to the substrates independently from microtubules. As for Aurora B, the localization seems to be more complicated. In vertebrates, each component of the chromosomal passenger complex containing Aurora B, Survivin, INCENP, and others was required for the proper localization of the other members [35,36]. Both Survivin and INCENP were responsible for targeting Aurora B at different stages in mitosis to act on different substrates. According to the latest report [24], Aurora C had a different N-terminal but the same localization with Aurora B. Our findings are not only in excellent agreement with this latest report, but we also proved that the novel Aurora C-SV with only 20 residues in N-terminal also localized and transferred in the same way as Aurora C during mitosis in HeLa cells. Therefore, the signals of the localizations for Aurora kinases might, to some extent, come from the binding to or interaction with the molecules specific to each family member. Since Aurora C was recently reported to be a novel chromosomal passenger protein, other members in this large complex may also facilitate the targeting and transferring of Aurora C. One challenge of future work would be identification of the factors that influence the cellular distribution of Aurora C and Aurora C-SV.

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References

[1] E.A. Nigg, A. Blangy, H.A. Lane, Dynamic changes in nuclear architecture during mitosis: on the role of protein phosphorylation in spindle assembly and chromosome segregation, Exp. Cell Res. 229 (1996) 174–180.
[2] D.O. Morgan, Cyclin-dependent kinases: engines, clocks, and micro-processors, Annu. Rev. Cell Dev. Biol. 13 (1997) 261–291.
[3] N.P. Pavletich, Mechanism of cyclin-dependent kinase regulation: structure of Cdks, their cyclin activators, and CIP and Ink4 inhibitors, J. Mol. Biol. 287 (1999) 821–828.
[4] J. Kuang, C.L. Ashorn, At least two kinases phosphorylate the MPM-2 epitope during *Xenopus* oocyte maturation, J. Cell Biol. 123 (1993) 859–868.
[5] E. Logarinho, C.E. Sunkel, The *Drosophila* POLO kinase localizes to multiple compartments of the mitotic apparatus and is required for the phosphorylation of MPM2 reactive epitopes, J. Cell Sci. 111 (1998) 2897–2909.
[6] T. Mayor, P. Meraldi, Y.D. Stierhof, E.A. Nigg, A.M. Fry, Protein kinases in control of the centrosome cycle, FEBS Lett. 452 (1999) 92–95.
[7] A.F. Straight, Cell cycle: checkpoint proteins and kinetochores, Curr. Biol. 7 (1997) 613–616.
[8] S.S. Taylor, E. Ha, F. McKeon, The human homologue of bub3 is required for kinetochore localization of bub1 and a Mad3/Bub1-related protein kinase, J. Cell Biol. 142 (1998) 1–11.
[9] C.S. Chan, D. Botstein, Isolation and characterization of chromosome-gain and increase-in-ploidy mutants in yeast, Genetics 135 (1993) 677–691.
[10] M. Carmena, C.W. Earnshaw, The cellular geography of Aurora kinases, Nature 4 (2003) 842–854.
[11] J.R. Bischoff, L. Anderson, Y.F. Zhu, K. Mossie, L. Ng, B. Souza, B. Schryver, P. Flanagan, F. Clairvoyant, C. Ginther, C.S.M. Chan, M. Novotny, D.J. Slamon, G.D. Plowman, A homologue of *Drosophila* aurora kinase is oncogenic and amplified in human colorectal cancers, EMBO J. 17 (1998) 3052–3065.
[12] L.E. Littlepage, H. Wu, T. Andresson, J.K. Deanehan, L.T. Amundadottir, J.V. Ruderman, Identification of phosphorylated residues that affect the activity of the mitotic kinase Aurora-A, Proc. Natl. Acad. Sci. USA 99 (2002) 15440–15445.
[13] M.Y. Tsai, C. Wiese, K. Cao, O. Martin, P. Donovan, J. Ruderman, C. Prigent, Y. Zheng, A Ran signaling pathway mediated by the mitotic kinase Aurora A in spindle assembly, Nat. Cell Biol. 5 (2003) 242–248.
[14] P.A. Eyers, E. Erikson, L.G. Chen, J.L. Maller, A novel mechanism for activation of the protein kinase aurora a, Curr. Biol. 13 (2003) 691–697.
[15] S. Chen, P. Chang, Y. Cheng, F. Tang, Y. Lin, Suppression of the STK15 oncogenic activity requires a transactivation-independent p53 function, EMBO J. 21 (2002) 4491–4499.
[16] P.D. Andrews, E. Knayko, W.J. Moore, J.R. Swedlow, Mitotic mechanics: the auroras come into view, Curr. Opin. Cell Biol. 15 (2003) 672–683.
[17] M. Shindo, H. Nakano, H. Kuroyanagi, T. Shirasawa, M. Mihara, D.J. Gilbert, N.A. Jenkins, N.G. Copeland, H. Yagita, K.

Okumura, cDNA cloning, expression, subcellular localization, and chromosomal assignment of mammalian aurora homologues aurora-related kinase (ARK) 1 and 2, Biochem. Biophys. Res. Commun. 244 (1998) 285–292.
[18] Y. Terada, Role of chromosomal passenger complex in chromosome segregation and cytokinesis, Cell Struct. Funct. 26 (2001) 653–657.
[19] T.C. Tseng, S.H. Chen, Y.P. Hsu, T.K. Tang, Protein kinase profile of sperm and eggs: cloning and characterization of two novel testis specific protein kinases (AIE1, AIE2) related to yeast and fly chromosome segregation regulators, DNA Cell Biol. 17 (1998) 823–833.
[20] M. Bernard, P. Sanseau, C. Henry, A. Couturier, C. Prigent, Cloning of Aurora C, a third human protein kinase related to *Drosophila* Aurora and budding yeast IPL1 that maps on chromosome 19q13.3-ter, Genomics 53 (1998) 406–409.
[21] M. Kimura, Y. Matsuda, T. Yoshioka, Y. Okano, Cell cycle-dependent expression and centrosome localization of a third human aurora/ipl1-related protein kinase, AIK3, J. Biol. Chem. 274 (1999) 7334–7340.
[22] N. Rompino, H. Yamamoto, Y. Ionov, Y. Li, H. Sawai, J.C. Reed, M. Perucho, Somatic frame shift mutation in BAX gene in colon cancers of microsatellite mutator phenotype, Science 14 (1997) 2195–2200.
[23] T. Takahashi, M. Futamura, N. Yoshimi, J. Sano, M. Katada, Y. Takagi, M. Kimura, T. Yoshioka, Y. Okano, S. Saji, Centrosomal kinases, HsAIRK1 and HsAIRK3, are overexpressed in primary colorectal cancers, Jpn. J. Cancer Res. 91 (2000) 1007–1014.
[24] X.Y. Li, G. Sakashita, H. Matsuzaki, K. Sugimoto, K. Kimura, F. Hanaoka, H. Taniguchi, K. Furukawa, T. Urano, Direct association with inner centromere protein (INCENP) activates the novel chromosomal passenger protein, Aurora C, J. Biol. Chem. 279 (2004) 47201–47211.
[25] S.K. Hanks, T. Hunter, The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification, FASEB J. 9 (1995) 576–596.
[26] S.K. Hanks, A.M. Quinn, T. Hunter, The protein kinase family: conserved features and deduced phylogeny of the catalytic domains, Science 241 (1988) 42–52.
[27] J.D. Thompson, T.J. Gibson, F. Plewniak, F. Jeanmougin, D.G. Higgins, Clustal X windows interface: flexible strategies for multiple sequences alignment aided by quality analysis tools, Nucleic Acids Res. 24 (1997) 4876–4882.
[28] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, Nucleic Acids Symp. Ser. 41 (1999) 95–98.
[29] Y. Yasui, T. Urano, A. Kawajiri, K. Nagata, M. Tatsuka, H. Saya, K. Furukawa, T. Takahashi, I. Izawa, M. Inagaki, Autophosphorylation of a newly identified site of Aurora-B is indispensable for cytokinesis, J. Biol. Chem. 279 (2004) 12997–13003.
[30] M. Huse, J. Kuriyan, The conformational plasticity of protein kinases, Cell 109 (2002) 275–282.
[31] R. Crane, B. Gadea, L. Littlepage, H. Wu, J.V. Ruderman, Aurora A, meiosis and mitosis, Biol. Cell 96 (2003) 215–229.
[32] A. Gil, How did alternative splicing evolve, Nature Rev. Genet. 5 (2004) 773–782.
[33] L.E. Littlepage, J.V. Ruderman, Identification of a new APC/C recognition domain, the A box, which is required for the Cdh1-dependent destruction of the kinase Aurora-A during mitotic exit, Genes Dev. 16 (2002) 2274–2285.
[34] R. Giet, C. Prigent, The non-catalytic domain of the *Xenopus laevis* aurora A kinase localizes the protein to the centrosome, J. Cell Sci. 114 (2001) 2095–2104.
[35] R.R. Adams, S.P. Wheatleya, A.M. Gouldsworthy, S.E. Kandels-Lewis, M. Carmena, C. Smythe, D.L. Gerloff, W.C. Earnshaw, INCENP binds the aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow, Curr. Biol. 10 (2000) 1075–1078.
[36] M.A. Bolton, W. Lan, S.E. Powers, M.L. McCleland, J. Kuang, P.T. Stukenberg, Aurora B kinase exists in a complex with survivin and INCENP and its kinase activity is stimulated by survivin binding and phosphorylation, Mol. Biol. Cell 13 (2002) 3064–3077.