CSC-1: a subunit of the Aurora B kinase complex that binds to the Survivin-like protein BIR-1 and the Incenp-like protein ICP-1

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The Aurora B kinase complex is a critical regulator of chromosome segregation and cytokinesis. In Caenorhabditis elegans, AIR-2 (Aurora B) function requires ICP-1 (Incenp) and BIR-1 (Survivin). In various systems, Aurora B binds to orthologues of these proteins. Through genetic analysis, we have identified a new subunit of the Aurora B kinase complex, CSC-1. C. elegans embryos depleted of CSC-1, AIR-2, ICP-1, or BIR-1 have identical phenotypes. CSC-1, BIR-1, and ICP-1 are interdependent for their localization, and all are required for AIR-2 localization. In vitro, CSC-1 binds directly to BIR-1. The CSC-1/BIR-1 complex, but not the individual subunits, associates with ICP-1. CSC-1 associates with ICP-1, BIR-1, and AIR-2 in vivo. ICP-1 dramatically stimulates AIR-2 kinase activity. This activity is not stimulated by CSC-1/BIR-1, suggesting that these two subunits function as targeting subunits for AIR-2 kinase.

Introduction

Accurate duplication and segregation of the genome and cellular organelles are accomplished through an exquisitely coordinated series of events. Execution of several mitotic processes and cytokinesis requires members of the Aurora kinase family, which is subdivided into three subfamilies, Aurora A, B, and C, based on their subcellular localization (Nigg, 2001). Aurora B has essential functions in meiotic and mitotic chromosome segregation, and cytokinesis.

Aurora B kinase concentrates on the subcellular structures that it regulates. During meiosis in Caenorhabditis elegans, Aurora B kinase localizes to a discrete region between homologous chromosomes in diakinesis and metaphase, where it acts to control chromosome segregation (Kaitna et al., 2002; Rogers et al., 2002). In mammalian cells, during prometaphase and metaphase, Aurora B concentrates in the inner centromeric region, a localization that may underlie its function in mitotic chromosome segregation (Martineau-Thuillier et al., 1998). After anaphase onset, Aurora B kinase localizes to the central spindle where it promotes the stable localization of the centralspindlin complex and therefore probably regulates cytokinesis (Kaitna et al., 2002; Mishima et al., 2002). One outstanding question is how this kinase becomes concentrated on these distinct structures at different moments during meiosis and mitosis.

Although the mechanism of targeting Aurora B kinase is not fully understood, its localization in several systems requires at least two other proteins, Incenp and Survivin/BIR-1. Depletion of Aurora B kinase, Incenp, or Survivin/BIR-1 causes identical phenotypes, and biochemical interactions have been detected between these three proteins (for review see Adams et al., 2001). Thus, Aurora B, BIR-1 (Survivin), and Incenp appear to form a complex, hereafter referred to as the ABI* complex.

Here we describe the discovery and characterization of an additional subunit of the Aurora B kinase complex, CSC-1. C. elegans hermaphrodites mutant for csc-1 produce embryos that are inviable due to defects in meiotic and mitotic chromosome segregation and cytokinesis; identical phenotypes are caused by depletion of AIR-2 (Aurora B), ICP-1 (Incenp), or BIR-1 (Survivin). We provide genetic, cell biological, and...
biochemical evidence that CSC-1 is a subunit of the Aurora B kinase complex.

Results and discussion

CSC-1 is a novel 27-kD protein required for chromosome segregation and cytokinesis

During a large-scale screen for maternal effect embryonic lethal mutations, we isolated a mutant allele (t1171) that exhibited the set of phenotypes characteristic of the ABI complex members (Fig. 1 A). In particular, meiotic chromosome segregation and polar body extrusion are defective. During mitosis, spindle assembly appears normal, but chromosome segregation fails entirely and a single large nucleus reassembles in the subsequent interphase. After spindle elongation, a cleavage furrow forms and ingresses, but it fails to ingress to completion, thus producing a multinucleate embryo.

The csc-1 locus maps to 16 cM on chromosome II. As the known members of the ABI complex map to other regions of the genome, this locus must be distinct, and it was named csc-1(t1171) for chromosome segregation and cytokinesis defective. The map position was refined by single nucleotide polymorphism (SNP) mapping to a small region containing 12 predicted genes, four of which were predicted to encode glutathione transferases (Fig. 1 B). The eight unique genes in the region were depleted by RNA interference (RNAi); depletion of one predicted gene product, Y48E1B.12, caused chromosome segregation and cytokinesis defects identical to those seen
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in csc-1(t1171) mutant embryos (Fig. 1 C). Chromosome behavior was analyzed using GFP–histone H2B in csc-1(RNAi) embryos. These embryos have defects in chromosome congression to the metaphase plate and in segregation of the chromosomes to the spindle poles. The condensed chromosomes do not form a well-ordered metaphase plate, and subsequently the chromatin becomes stretched along the spindle axis, and cytokinesis initiates but ultimately fails (Fig. 1 C). These phenotypes are identical to those observed in embryos depleted for ICP-1 (Fig. 1 C) and AIR-2 (Kaitna et al., 2000).

The csc-1 locus is predicted to encode a 27-kD protein with a potential coiled coil in the NH2 terminus. No other conserved domains were detected. This protein also contains an imperfect direct repeat of 23–25 residues (Fig. 1 D). Surprisingly, with the exception of a sequence from the C. briggsae genome, no other sequences in the available databases have significant homology to CSC-1. To confirm the identity of csc-1, the gene was sequenced from animals homozygous for csc-1(t1171), and a single point mutation was identified that changes glutamine 128 into a stop codon. In addition, a 2.6-kb genomic fragment can rescue csc-1(t1171) to fertility. Worms homozygous for the strong loss of function allele of csc-1(t1171) are viable and semi-sterile, producing few embryos, all of which are inviable (average brood size of dpy-2[e8] is 51.2 [n = 13]; for dpy-2[e8];csc-1[t1171] the value is 7.2 [n = 105]). The viability and incomplete sterility of csc-1(t1171) animals may result from perdurance of the maternally provided protein.

CSC-1 localizes to meiotic and mitotic chromosomes and to the central spindle during anaphase

CSC-1, BIR-1, and ICP-1 are mutually dependent for their localization and are all required for AIR-2 localization

Figure 2.

CSC-1 localizes in a similar manner as ABI complex members. CSC-1 localization in wild-type embryos was analyzed by immunofluorescence. Staining is observed in the central region of meiotic bivalents during diakinesis (A) and in prometaphase (B) of meiosis I. At anaphase I, CSC-1 localizes to the central region of the meiotic spindle (C). During mitotic metaphase, CSC-1 localizes to the chromosomes (D), and in anaphase, it localizes to the central spindle (E). The CSC-1 signal on the central spindle is wider than that of the centralspindlin component ZEN-4 (F). The above-mentioned staining patterns are absent in csc-1(RNAi) embryos (G); anti-tubulin staining demonstrates antibody accessibility (H).
dent, for their proper localization (Fig. 3 A, fourth row; Spe- 
eliotes et al., 2000; unpublished data). Thus, CSC-1, BIR-1, 
and ICP-1 are mutually interdependent, but AIR-2 indepen-
dent, for their localization to chromosomes. AIR-2 requires 
CSC-1, BIR-1, and ICP-1 to localize to chromosomes.

To examine whether these localization dependencies may 
also be due to an effect on protein stability, we performed 
Western blots on embryos isolated from RNAi-treated 
worms and assessed the levels of ICP-1, AIR-2, CSC-1, and 
BIR-1. Depletion of CSC-1 and ICP-1 reduced the levels of 
BIR-1 significantly, whereas depletion of BIR-1 and AIR-2 
did not greatly affect the accumulation of the other subunits 
(Fig. 3 B). These results suggest a functional interaction be-
tween CSC-1 and BIR-1.

CSC-1 associates with ABI complex members in vivo 
and binds to BIR-1 in vitro

As CSC-1, BIR-1, and ICP-1 are interdependent for their 
localization, these proteins likely form a protein complex in 
vivo. To examine whether such a complex exists, we used 
Western blotting to determine if anti–CSC-1 immunopre-
cipitates (IPs) contain ABI complex members. Indeed, both 
BIR-1 and ICP-1 associate with CSC-1 in C. elegans embryo 
extracts (Fig. 4 A). We conclude that in vivo, CSC-1 is a 
member of the ABI complex.

The results above suggest that CSC-1 might directly bind 
to one of the members of this complex. To examine this pos-
sibility, binding assays were performed using in vitro–trans-
lated proteins. CSC-1 and BIR-1 associate in the absence of 
any other nematode proteins (Fig. 4 B). The baculovirus 
IAP repeat (BIR) domain of BIR-1 is stabilized by a Zn^{2+} 
atom that is coordinated by three cysteine residues and a his-
tidine. The interaction between CSC-1 and BIR-1 is abol-
ished when Zn^{2+} is chelated with 1,10-O-phenanthroline 
and restored by addition of excess zinc. In addition, muta-
tion of BIR-1 at cysteine 83, one of the residues predicted to 
chelate zinc, to alanine, abolishes the interaction between 
CSC-1 and BIR-1 (Fig. 4 B). We conclude that the interac-
tion between CSC-1 and BIR-1 requires a native, Zn^{2+}- 
bound BIR domain. Furthermore, as several studies indi-
cate that recombinant Survivin forms a stable dimeric struc-
ture (Shi, 2000), we investigated whether CSC-1 binds 
to a dimer of BIR-1 and whether the residues implicated 
in dimer formation are critical for the interaction be-
tween CSC-1 and BIR-1. We have not observed any indica-
tions that BIR-1 is a dimer when bound to CSC-1 (see 
Fig. S1, available at http://www.jcb.org/cgi/content/full/
jcb.200207117/DC1).

Efficient binding of the CSC-1/BIR-1 subcomplex to 
ICP-1 and formation of a multiprotein complex that 
activates AIR-2 kinase activity

We next investigated whether BIR-1, CSC-1, or the BIR-1/ 
CSC-1 complex biochemically interacts with ICP-1 and 
AIR-2 kinase. Cell lysates were prepared from insect cells 
infected with baculoviruses expressing the individual pro-
teins. Using chitin-binding domain (CBD)–tagged ICP-1, 
we examined whether a complex could be formed between 
ICP-1 and CSC-1 or BIR-1 by mixing these lysates and re-
covering the tagged ICP-1 on chitin beads. In these binary
binding assays, no detectable interactions were found between ICP-1 and CSC-1; a weak but reproducible interaction was observed between ICP-1 and BIR-1. However, when lysates containing all three subunits were mixed, robust formation of an ICP-1/BIR-1/CSC-1 complex was observed (Fig. 5 A). These data, combined with the previous observation of a robust BIR-1/CSC-1 complex, suggest that ICP-1 binds strongly to this subcomplex, but not to the individual subunits.

Our previous studies showed that the COOH-terminal conserved domain of ICP-1 is sufficient to bind to AIR-2 (Kaitna et al., 2000). We therefore tested whether AIR-2 also binds directly to CSC-1 or BIR-1 or whether the presence of CSC-1/BIR-1 alters the binding of AIR-2 to ICP-1.
Binary binding assays using epitope-tagged ICP-1 or AIR-2 confirmed the previously established interaction between ICP-1 and AIR-2, but no significant interactions were observed between AIR-2 and CSC-1 or BIR-1 (Fig. 5, A and B). Moreover, AIR-2 did not bind appreciably to the CSC-1/BIR-1 complex, except in the presence of ICP-1 (Fig. 5 B). In addition, the presence or absence of CSC-1/BIR-1 did not significantly affect the interaction between ICP-1 and AIR-2. The binding of AIR-2 to BIR-1 required CSC-1 (and ICP-1) and vice versa. Thus, AIR-2 can associate with the CSC-1/BIR-1 subcomplex, but this interaction is likely indirect via ICP-1.

The CSC-1/BIR-1/ICP-1 complex associates with AIR-2, raising the possibility that it regulates its activity. AIR-2 is not active above background levels, using myelin basic protein (MBP) as a substrate, when expressed alone in insect cells. Mixing of AIR-2–containing lysates with CSC-1, BIR-1, or both did not activate AIR-2 kinase. However, AIR-2 was significantly activated upon mixing with an ICP-1–containing lysate (Fig. 5 B). We next assessed whether CSC-1 or BIR-1, either alone or in combination, affected the ICP-1–activated kinase activity. We did not observe a significant, reproducible change in the ICP-1–stimulated AIR-2 kinase activity. These data confirm earlier reports that ICP-1 is an activator of AIR-2 (Kang et al., 2001; Bishop and Schumacher, 2002) and suggest that CSC-1 and BIR-1 do not function primarily by regulating AIR-2 kinase activity, at least toward the model substrate MBP.

AIR-2/ICP-1/CSC-1/BIR-1 form a robust complex, which was purified to obtain an indication of the relative abundance of each subunit in the complex. We coinjected insect cells with viruses that express CBD–AIR-2, ICP-1, CSC-1, and BIR-1 and purified AIR-2 and associated proteins by chitin affinity chromatography. The complex was eluted from chitin beads using the tobacco etch virus (TEV) protease to cleave a recognition site that was engineered between the CBD tag and AIR-2. These preparations contained approximately equal levels of CSC-1, BIR-1, and ICP-1 and slightly more AIR-2 (which carried the affinity tag) (Fig. 5 D). This complex has high kinase activity against MBP. We also purified a complex containing only AIR-2 and ICP-1. The kinase activity of both preparations paralleled the amount of AIR-2 and ICP-1, again indicating that CSC-1 and BIR-1 do not greatly affect AIR-2 kinase activity (Fig. 5 D).

Composition and function of the ABI complex
Genetic, cell biological, and biochemical assays indicate that CSC-1 functions as an essential subunit of the ABI kinase complex. This complex mediates chromosome segregation and cytokinesis in C. elegans and many other eukaryotic organisms. To understand these fundamental cellular events, it is critical to understand the function of this kinase complex and identify its important substrates. Furthermore, it is important to investigate the enzyme itself by characterizing its composition, establishing how it localizes, and deciphering how its activity is regulated. By genetic and cell biological criteria, CSC-1 is essential for all known functions of the Aurora B kinase, AIR-2. CSC-1 is required for AIR-2 localization, and the cellular consequences of CSC-1 depletion are identical to that of AIR-2. However, biochemical analysis indicates that AIR-2 kinase activity in vitro does not require CSC-1. Thus, we suggest that a complex containing CSC-1/BIR-1/ICP-1 functions to localize and activate AIR-2, the activation function being provided principally by ICP-1.

Conservation of CSC-1
Whereas AIR-2, BIR-1, and ICP-1 have well-defined orthologues in many other eukaryotes, we have been unable to detect proteins with significant similarity to CSC-1 in any other organism except for C. briggsae. One possibility is that CSC-1 is unique to nematodes. Alternatively, our failure to identify an orthologue of CSC-1 could be due to weak conservation at the primary sequence level. Finally, CSC-1 function might be provided by one of the previously characterized members of the ABI complex in other organisms. Vertebrate Survivin has been reported to bind directly to Incenp (Wheatley et al., 2001), the NH₂ terminus of Incenp is sufficient to interact with Survivin in a cell extract system (Bolton et al., 2002), and affinity purification of SlI5p reveals coassociation of Ipl1p and Bir1p (the budding yeast orthologues of ICP-1, AIR-2, and BIR-1, respectively) (Cheeseman et al., 2002). In addition, interactions have been detected between Survivin and Aurora B (Wheatley et al., 2001; Bolton et al., 2002; Chen et al., 2003). In contrast, we have found that stable interaction of ICP-1 and BIR-1 as well as AIR-2 and BIR-1 requires CSC-1 (ICP-1 is also required in the latter case). A simple model is that the function of Incenp has diverged into two polypeptides, ICP-1 and CSC-1, at some point during evolution. However, we have not detected significant primary sequence conservation between CSC-1 and the NH₂-terminal domain of vertebrate Incenp, though both proteins do contain regions predicted to form a coiled coil.

Although the split gene model for csc-1 is speculative, it is not without precedent. For example, in most organisms, separase is an inactive dimeric complex that is activated by degradation of its partner, securin. In Drosophila, however, separase is an inactive trimer that is activated by degradation of one subunit (Jager et al., 2001).

Subcellular targeting of the ABI complex
Our results provide some insight as to how the ABI complex concentrates on the structures it regulates in a cell cycle–dependent fashion, namely meiotic and mitotic chromosomes and the central spindle. The interdependence of BIR-1, ICP-1, and CSC-1 for centromeric localization (as well as to the central spindle) indicates that the binding sites for the ABI complex depend on all three (and perhaps additional) subunits. Binding of the ABI complex to chromosomes is dependent upon the integrity of the cohesin complex (Sonoda et al., 2001; Kaitna et al., 2002). This dependency is intriguing because one function that has been suggested for the ABI complex is to regulate microtubule–kinetochore interactions in response to tension exerted between associated sister chromatids (Kaitna et al., 2002; Tanaka et al., 2002).

The other prominent site of localization of the ABI complex is the central spindle. Notably, there are at least two distinct domains within the central spindle, a tightly restricted one that contains the centralspindlin complex and a some-
what broader domain in which the ABI complex localizes. Whereas stable localization of the centralspindlin complex requires the ABI complex (Kaitna et al., 2000), localization of the ABI complex is largely independent of centralspindlin (Jantsch-Plunger et al., 2000). Evidence is accumulating that posttranslational modification of the ABI complex regulates its redistribution to the spindle midzone. Aurora B kinase activity is required for the release of ABI complex members from chromosomes during anaphase and for association of the ABI complex with the central spindle (Severson et al., 2000; Murata-Hori and Wang, 2002; unpublished data).

Concluding remarks

The results presented here establish that the Aurora B kinase associates with a multisubunit targeting complex composed of CSC-1, BIR-1, and ICP-1. ICP-1 binds to the CSC-1/BIR-1 complex and to AIR-2; this latter interaction activates the catalytic activity of AIR-2 (Kang et al., 2001; Bishop and Schumacher, 2002; this paper). It remains to be determined how the BIR-1/CSC-1/ICP-1 subcomplex localizes to chromosomes and the central spindle, thereby recruiting Aurora B to these sites. CSC-1 and BIR-1 may have additional functions, such as regulating the substrate specificity of Aurora B.

Materials and methods

Worm strains and alleles

Strains used in this study include GE896 dpy-2(eh) csc-1(t11717)/mnC1 [II], him-3(e1147), MG303 nls32[pA2132::p-1/GFP/histoneH2B] III (derived from AZ212), Bristol N2, and CB4856, which were obtained from the Caenorhabditis Genetics Center.

Genetic mapping and genomic rescue

csc-1 was mapped to 16 cM on LGII. Fine mapping was performed using SNPs in crosses of GE896 with CB4856 (SNP data and primer sequences available on request). A 2.6-kb genomic fragment was co-injected with L4440 (provided by Andy Fire, Carnegie Institution of Washington, Baltimore, MD), transformed into HT115(DE3), and grown overnight. Nematode growth plates containing IPTG and ampicillin were seeded with these cultures for 24 h at 20°C. L4 animals were grown on these plates for 2 d at 20°C before analysis.

RNAi

Target sequences were PCR amplified from C. elegans cDNA, cloned into L4440 (provided by Andy Fire, Carnegie Institution of Washington, Baltimore, MD), transformed into HT115(DE3), and grown overnight. Nematode growth plates containing IPTG and ampicillin were seeded with these cultures for 24 h at 20°C. L4 animals were grown on these plates for 2 d at 20°C before analysis.

Immunostaining and microscopy

Immunostaining and microscopy were performed as previously described (Kaitna et al., 2002). Antibody sources and dilutions were as follows: Rab anti-phos-H1 (Sigma) (Upstate Biotechnology), 1:500; Rab anti-CSC-1,1:750; Rab anti-AIR-2, 1:750; Rab anti-BIR-1 (Speliotes et al., 2000), 1:100; Rab anti-ICP-1 (Oegema et al., 2001), 1 mg/ml; Mo anti-GFP antibodies (Roche), 1:100; and rat YOL1/34 anti-tubulin monoclonal antibody, 1:300.

Anti-CSC-1 antibodies

CSC-1-specific antiserum were produced in rabbits (Garmsch Laboratories) using a COOH-terminal peptide (CGPGLOPETENAGTSV) coupled to keyhole limpet hemocyanin. An antibody raised against an NH2-terminal peptide provided essentially identical results in immunofluorescence and immunoprecipitation assays. Antibodies were affinity purified using immobilized peptides.

Embryo extract preparation

1 ml of C. elegans embryos obtained by alkaline hypochlorite treatment of gravid hermaphrodites was diluted in 1 ml buffer A (20 mM Heps, 50 mM NaCl, 2 mM MgCl2, 5 mM EGTA, and 0.5% Triton X-100) and frozen in liquid nitrogen. The frozen pellets were ground to powder in a liquid nitrogen-cooled mortar. The powder was diluted in 1 ml buffer A and centrifuged at 20,000 g for 10 min at 4°C.

In vitro binding assays

Proteins were expressed in vitro with the TNT reticulocyte lysate system (Promega) in the presence of [35S]methionine and cysteine (Translabel; ICN Biomedicals). After translation, 10 l of each translation product was diluted in 80 l buffer A and incubated at 4°C for 30 min. Where indicated, EDTA was added to 10 mM, 1,10-phenanthroline 2 mM, and ZnCl2, 5 mM. Proteins were immunoprecipitated or affinity purified with chin beads. Proteins bound to the beads were analyzed by SDS-PAGE followed by autoradiography with a phosphoimager.

Expression of recombinant proteins and kinase assay

Recombinant baculoviruses were produced in Sf9 cells using the Bac-to-Bac system (Invitrogen). Recombinant proteins were expressed in High Five cells. Cells were harvested 48 h after viral infection and lysed in 20 mM Heps, pH 7.5, 150 mM NaCl, 5 mM EGTA, 1 mM MgCl2, 1 mM PMSF, 0.1% Triton X-100, 5 mM β-mercaptoethanol, 10 μg/ml leupeptin, 10 μg/ml pepstatin. The lysates were centrifuged at 15,000 g for 10 min at 4°C. For binding experiments, equal volumes of the extracts were mixed in the indicated combinations (control extracts were substituted where appropriate) and recovered by overnight binding to chin beads. Beads were washed in lysin buffer or kinase buffer and assayed for Western blotting or MBP kinase activity.

Soluble purified recombinant protein complexes were obtained by co-expression of insect cells with the indicated proteins, and the purified complexes were eluted from chin beads by incubation with tobacco etch virus (TEV) protease. Eluates were visualized by SDS-PAGE, and ~20-60 mg of protein was assayed for kinase activity.

Kinase reactions were performed for 20 min at 20°C in kinase buffer (20 mM Heps, pH 7.5, 150 mM NaCl, 10 mM MgCl2, 1 mM DTT, 0.5 μM okadaic acid, 50 μM ATP, 50 μCi/μl [γ-32P]ATP [NEN Life Science Products], 250 ng/μl MBP). Reactions were separated by SDS-PAGE, and radioactive phosphate incorporation was visualized by phosphoimaging.

Online supplemental material

A supplemental figure (Fig. S1) is available at http://www.jcb.org/cgi/content/full/jcb.200207117/DC1. This figure provides evidence that formation of the CSC-1/BIR-1 complex is insensitive to mutations of residues suggested to be involved in dimerization of the BIR-1 orthologue, Survivin.

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