Role of Chromosomal Passenger Complex in Chromosome Segregation and Cytokinesis

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ABSTRACT. Chromosomal passenger proteins associate with chromosomes early in mitosis and transfer to the spindle at ana/telophase. Recent results show that aurora B/AIM-1 (aurora and Ipl1-like midbody-associated protein kinase), which is responsible for mitotic histone H3 phosphorylation, INCENP (Inner Centromere protein) and Survivin/BIR are in a macromolecular complex as novel chromosomal passenger proteins. Aurora B/AIM-1 can bind to Survivin and the C-terminal region of INCENP, respectively, and colocalizes with both proteins to the centromeres, midzone and midbody. Disruption of either aurora B/AIM-1 or INCENP function leads to severe defects in chromosome segregation and cytokinesis. Moreover, the formation of the central spindle through anaphase to cytokinesis is also disrupted severely.

These data suggest that chromosomal passenger complex is required for proper chromosome segregation by phosphorylating histone H3, and cytokinesis by ensuring the correct assembly of the midzone and midbody microtubule. Chromosomal passenger protein complex may couple chromosome segregation with cytokinesis.

Key words: chromosomal passenger/aurora B/AIM-1/INCENP/survivin/cytokinesis/chromosome segregation

Cytokinesis begins shortly after chromosome segregation, when the proposed interaction of the microfilament and microtubule mitotic spindle leads to the formation of the actomyosin contractile ring at the equatorial cortex. Although many genes have been involved in chromosome segregation and cytokinesis, the exact mechanism by which these processes are regulated was unknown. Recently, chromosomal passenger proteins have become well known as key factors in both processes.

Chromosomal passenger proteins are characterized by the unique property of translocation from chromosome to central spindle at anaphase onset. *INCENP* (Inner Centromere Protein) was initially characterized as a chromosomal passenger protein (Cooke *et al.*, 1987). A second passenger protein, TD-60 (telophase disc antigen), is also an early marker of furrow assembly. Recently, AIM-1 was identified as a mammalian homologue of aurora B that regulates chromosome segregation in yeast (Terada *et al.*, 1998; Tatsuka *et al.*, 1998). AIM-1 behaves as a so-called chromo-

somal passenger protein and relocalizes to the telophase disc at anaphase. In addition, survivin/Bir-1 was identified as a chromosomal passenger.

Chromosomal passenger protein may be to integrate the chromosomal and cytokinesis events of mitosis.

Mammalian aurora B kinase/AIM-1

Aurora kinases have been named after the *Drosophila* kinase aurora (Glover *et al.*, 1995). They belong to a multigenic family of mitotic kinases with at least three members in mammalian cells (Giet and Prigent, 1999; Bischoff and Plowman, 1999).

Mammalian aurora B kinase/AIM-1 was first cloned in a screen to isolate novel protein kinases that caused growth arrest when overexpressed in *S. cerevisiae* (Terada *et al.*, 1998). AIM-1 behaves as a chromosomal passenger protein: in prometaphase, its signal is weak, but it localizes in centrosomes. During prometaphase-metaphase, AIM-1 associates with chromosomes. In anaphase, AIM-1 transfers from chromosomes to central spindles. In telophase, AIM-1, eventually, becomes more sharply concentrated at the midbody (Terada *et al.*, 1998; Bischoff *et al.*, 1998).

Kinase-inactive form in AIM-1 blocked cytokinesis. Disruption of AIM-1 function has profound effects on the for-

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Abbreviations: AIM-1, aurora and Ipl1-like midbody-associated protein kinase; INCENP, Inner Centromere protein.

mation of the central spindle through anaphase to cytokinesis. In these cells, cleavage furrow formation was inhibited severely. Eventually, these cells become polyploid (Terada *et al.*, 1998; Tatsuka *et al.*, 1998). These results are strikingly similar to those found on disruption of the *C. elegans* aurora B homologue *air-2* (Shumacher *et al.*, 1998). RNAi of AIR-2 resulted in normal cleavage furrow formation, but the furrows regressed and did not complete cytokinesis.

During the last phase of megakaryopoiesis, megakaryocyte undergoes polyploidization, which is characterized by DNA duplication without cytokinesis. Interestingly, AIM-1 transcription is repressed during megakaryocytic polyploidization of megakaryocytic cell lines. Furthermore, overexpression of AIM-1 gene canceled polyploidization significantly. These data indicate that downregulation of aurora B/AIM-1 kinase couples with megakaryocytic polyploidization of hematopoietic cells (Kawasaki, *et al.*, 2000). These results suggest that members of the aurora B kinase are required in late mitosis for formation and/or stabilization of cleavage furrow and the completion of cytokinesis.

Aurora B as a mitotic histone H3 kinase

During cell division, phosphorylation of histone H3 typically takes place within the condensed chromatin. A strong correlation exists between the phosphorylation of histone H3 and mitotic chromosome condensation and proper chromosome segregation (Cheung *et al.*, 2000; de la Barre *et*

al., 2000). Histone H3 is not phosphorylated during interphase but becomes phosphorylated at Ser 10 just prior to metaphase, consistent with its role in a late stage of chromosome condensation (Bradbury 1992; Hendzel *et al.*, 1997). Indeed, mutation of the mitotic phosphorylation site of histone H3 of *Tetrahymena* leads to both chromosome condensation and segregation defect (Wei *et al.*, 1999).

Recent findings suggest that aurora B family and NIMA kinase in *Aspergillus nidulans* (De Souza *et al.*, 2000) are responsible for histone H3 phosphorylation. The enzyme required for histone H3 phosphorylation in *S. cerevisiae* is the Ipl1 (for increased in ploidy). In yeast, the aurora B/Ipl1 is involved in cell cycle progression and more particularly in the segregation and disjunction of chromosomes (Chan and Botstein, 1993; Biggins *et al.*, 1999). Moreover, *C. elegans* *air-2* and *Drosophila* aurora B protein kinase were shown to have the same function (Hsu *et al.*, 2000; Giet *et al.*, 2001). Overexpression of mammalian AIM-1 kinase-negative gene inhibited mitotic histone H3 phosphorylation (Tatsuka *et al.*, submitted). Taken together, the above data demonstrates that aurora B is essential for chromosome segregation by phosphorylating mitotic histone H3 as one of histone H3 kinase (Fig. 1).

INCENP (Inner centromere protein)

The INCENP is required for normal chromosome segregation and completion of cytokinesis in *S. cerevisiae*, *X. laevis*, *C. elegans*, *D. melanogaster*, and mammalian

Fig. 1. Multiple functions in chromosomal passenger complex. Aurora B/AIM-1 phosphorylates histone H3 on Ser10 in complex with INCENP and survivin. Mitotic histone H3 phosphorylation is required for chromosome condensation (1) and proper segregation (3) by recruitment of condensin (2) (Giet, *et al.*, 2001). Chromosomal passenger complex transfers to the central spindle midzone during anaphase and telophase. Aurora B/AIM-1 is required for formation and/or stabilization of central spindles in cytokinesis (4).

cells. (Kaitna *et al.*, 2000; Adams *et al.*, 2000; Adams *et al.*, 2001; Mackay *et al.*, 1998).

It was shown that INCENP is stored as a complex with aurora B in *Xenopus* eggs (Adams *et al.*, 2000), *C. elegans* (Katina *et al.*, 2000) and mammalian cells (Tatsuka *et al.*, submitted). The C-terminal domain of INCENP can bind to aurora B/AIM-1 directly. At mitosis, INCENP is carried by chromosomes on the metaphase plates, where it is relocated on the centromeres as metaphase proceeds. At anaphase this protein migrates to the central spindles. In mammalian cells, a very precise colocalization of INCENP and aurora B/AIM-1 was found (Mackay *et al.*, 1998; Terada *et al.*, 1998). Moreover, the localization of aurora B/AIM-1 depends on that of INCENP and the perturbation of INCENP localization is similarly accompanied by that of aurora B/AIM-1 (Adams *et al.*, 2001; Tatsuka *et al.*, submitted). Expression of dominant-negative INCENP mutant caused a failure in cytokinesis. In tissue culture cells containing the INCENP mutant protein, the furrows formed but regressed (Mackay *et al.*, 1998; Eckley *et al.*, 1997). INCENP is required for targeting of aurora B/AIM-1 to centromeres and

midzone (Fig. 1), because proper localization of aurora B/AIM-1 was perturbed in the cells lacking INCENP function (Adams *et al.*, 2001; Tatsuka *et al.*, submitted).

Survivin/Bir family

Survivin, a baculovirus inhibitor of apoptosis (IAP) repeat (BIR) motif protein (Ambrosini *et al.*, 1997), is a third passenger protein. Because of its BIR motif, survivin is a potential member of the IAP family of proteins, which act at discrete steps to regulate the apoptotic pathway of cell death (Deveraux and Reed, 1999). In *C. elegans*, BIR-1 protein acts as a passenger protein, first localizing to the chromosomes, and migrating to the midbody during cleavage (Speilotes *et al.*, 2000). In addition, BIR-1 functions in concert with AIR-2 by binding to it directly during cell division. In *C. elegans*, *bir-1* (RNAi) embryos exhibited defects in chromosome alignment, segregation and cytokinesis. Air-2 failed to associate with chromosomes and mitotic H3 phosphorylation was also inhibited in the *bir-1* (RNAi) embryos. The same defects were observed in *air-2* (RNAi)

Cytokinesis Function of Chromosomal Passenger Complex

Fig. 2. Cytokinesis function of chromosomal passenger complex. Speculative model in which chromosomal passenger complex regulates the formation and/or stabilization of central spindles. The chromosomal passenger complex transfers to the central spindles through INCENP binding to microtubules. INCENP is required for aurora B/AIM-1 targeting, and that either or both of these proteins may be required for targeting of the CHO1/MKLP-1 motor protein (Nislow *et al.*, 1992; Kuriyama *et al.*, 1994; Raich *et al.*, 1998; Adams *et al.*, 1998; Powers *et al.*, 1998). The CHO1/MKLP-1 protein interacts with the Cyk-4 (Rho-GTPase-activating protein) (Kaitna, *et al.*, 2000). Aurora B/AIM-1, INCENP, survivin and CHO1/MKLP-1 are all mutually interdependent for stable targeting to the central spindle.

embryos. Thus, survivin/BIR-1 forms a complex with aurora B and localize it to chromosomes and midzone (Fig. 1).

Role of chromosomal passenger and kinesin motor protein in cytokinesis

Several studies of mammalian kinesin motor protein CHO1/MKLP1, *Zen 4* (the *C. elegans* CHO-1/MKLP1 homologue) and *pavarotti* (the *Drosophila* CHO-1/MKLP1 homologue) have suggested involvement of this kinesin motor protein in cytokinesis (Nislow *et al.*, 1992; Kuriyama *et al.*, 1994; Raich *et al.*, 1998; Adams *et al.*, 1998; Powers *et al.*, 1998).

In *pavarotti*, microtubules failed to bundle in the central spindle and components of the contractile ring failed to assemble, resulting in a cytokinesis failure. The ability of the motor protein to organize central spindles and the midbody matrix appears to be essential for the cytokinesis function.

Cells that completed cytokinesis formed strong microtubule bundles containing INCENP and CHO1/MKLP1, eventually resulting in the production of a midbody. On the other hand, cells that were unable to cleave failed to assemble robust microtubule bundles and lacked INCENP and CHO-1/MKLP1 in the midzone (Savoian *et al.*, 1999).

Importantly, the cytokinesis defects in cells lacking either *air-2* (the *C. elegans* aurora B homologue) or *ICP-1* (the *C. elegans* INCENP homologue) may be caused by disrupted localization of the midzone motor protein, *Zen-4/CeMKLP-1* (Kaitna *et al.*, 2000). These data suggest that INCENP is required for aurora B/*air-2* targeting, and that either or both of these proteins may be required for targeting of the MKLP-1 motor protein.

Aurora B/AIM-1 kinase may modify substrates, such as CHO-1/MKLP1 or other midbody proteins, to promote the assembly of the midzone through microtubule crosslinking or transport of factors required to stabilize the midzone (Fig. 2).

Summary

Nuclear division and cytokinesis are normally coordinated in the cell cycle, and cytokinesis occurs exactly after completion of chromosome segregation. Errors of this order result in aneuploidy, which can lead to cell death or oncogenesis (Tatsuka *et al.*, 1998; Terada *et al.*, 1995). This implies the existence of a checkpoint control that suppresses cytokinesis before completion of chromosome segregation. The chromosomal passenger proteins are involved in chromosome segregation by mitotic phosphorylation of histone H3, and cytokinesis by ensuring the correct assembly of the midzone and midbody microtubule. One possibility is that the primary role of chromosomal passenger proteins is to link chromosome segregation to cytokinesis. Further observations on chromosome passenger complex may lead to a

model for the way cytokinesis is coordinated with chromosome segregation.

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