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COMMENTARY

Aurora/Ipl1p-related kinases, a new oncogenic family of mitotic serine-threonine kinases

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SUMMARY

During the past five years, a growing number of serine-threonine kinases highly homologous to the *Saccharomyces cerevisiae* Ipl1p kinase have been isolated in various organisms. A *Drosophila melanogaster* homologue, aurora, was the first to be isolated from a multicellular organism. Since then, several related kinases have been found in mammalian cells. They localise to the mitotic apparatus: in the centrosome, at the poles of the bipolar spindle or in the midbody. The kinases are necessary for completion of mitotic events such as centrosome separation, bipolar spindle assembly and chromosome segregation. Extensive research is now focusing on these proteins because the three human homologues are overexpressed in various primary cancers. Furthermore, overexpression of one of these kinases transforms cells. Because of the myriad of kinases identified, we suggest a generic name: Aurora/Ipl1p-related kinase (AIRK). We denote AIRKs with a species prefix and a number, e.g. HsAIRK1.

Key words: Kinase, Aurora, Mitosis, Spindle, Oncogene

INTRODUCTION

During cell cycle progression in higher eukaryotes, mitosis is a critical phase, because, upon cell division, the two daughter cells must inherit the same genetic background. Although mitosis is the shortest phase of the cell cycle, it is then that the cell undergoes the most rapid and dramatic structural re-organisations. The interphase microtubule network depolymerises between the end of G_2 phase and the beginning of prophase (Verde et al., 1990; Aizawa et al., 1991; Zhai et al., 1998). Centrosomes, which are duplicated at the end of S phase, migrate around the nucleus. Upon entry into prophase, chromatin condenses, and the nuclear envelope breaks down. During prometaphase, the microtubules forming the bipolar spindle tend to nucleate from separated centrosomes that act as the two microtubule-organising centres (MTOCs). In metaphase, the kinetochores capture the plus-ends of microtubules and facilitate the alignment of the chromosomes at the metaphase plate in the centre of the mitotic spindle. Two identical sets of chromosomes start to separate in anaphase A. This movement is generated by a decrease in the length of the kinetochore fibres of microtubules (Desai et al., 1998). The separation of the chromosomes increases in anaphase B because of the migration of the spindle poles, which is probably caused by the gliding of polar microtubules. Motor proteins such as dynein, kinesins and kinesin-related proteins participate in these different dynamic mechanisms (Gaglio et al., 1996; Stearns, 1997; Walczak et al., 1998).

All the above events are tightly regulated through the balance between phosphorylation and dephosphorylation reactions (Nigg et al., 1996). When the cell enters mitosis, the phosphorylation states of numerous proteins dramatically change, which is demonstrated by the use of MPM-2 antibodies raised against epitopes that have been specifically phosphorylated in mitosis (Davis et al., 1983). The centrosomes and the mitotic spindle are particularly enriched with MPM-2 epitopes (Vandre and Borisy, 1989; Centonze and Borisy, 1990). Two protein kinases are known to phosphorylate MPM-2 epitopes in mitosis: CDK1 ($p34^{cdc2}$) and PLK1 (polo-like kinase 1) (Kuang and Ashorn, 1993; Logarinho and Sunkel, 1998). The CDK1-cyclinB complex triggers entry into mitosis, and its regulation is now well understood. Substrates for both kinases include motor proteins (Liao et al., 1994; Blangy et al., 1995, 1997), components of the nuclear envelope (Peter et al., 1990) and cell cycle regulators (Patra and Dunphy, 1998; Kumagai and Dunphy, 1998; Hoffman et al., 1993). Polo-like kinases (PLKs) were named after the *Drosophila* Polo kinase (Glover et al., 1995). Members of this kinase family range from the yeast proteins (the *S. cerevisiae* Cdc5p (Hartwell and Smith, 1985) and *S. pombe* Plo1p (Ohkura et al., 1995)) to human PLK1 (Golsteyn et al., 1995). The kinase localises to spindle-pole bodies in prophase, the poles of the mitotic spindle, and the kinetochores in metaphase. In

anaphase and telophase, during chromosome segregation, the protein re-localises on the central spindle in the midbody. Relocalisation of PLKs during mitosis is associated with different functions of the kinase in spindle formation, anaphase and cytokinesis. The kinase is involved in several regulation processes, such as the autoamplification loop that triggers entry into mitosis and activation of the anaphase-promoting complex (APC), which triggers exit from mitosis. Moreover, the association of PLKs with members of the KLP (kinesin-like protein) family suggests that the kinase regulates the function of motor proteins during mitosis (Heidi and Nigg, 1997; Glover et al., 1998, for review).

Recently, a new family of mitotic kinases that includes the yeast *IPL1* (for increase in ploidy 1) and the *Drosophila Aurora* gene products has been identified. The functions of these kinases are closely linked to microtubule dynamics. The kinases associate with the centrosome around the pericentriolar material, as well as the microtubules at the bipolar mitotic-spindle poles and the midbody microtubules. The *S. cerevisae* genome encodes only one such kinase (Goffeau et al., 1996; Hunter and Plowman, 1997): Ipl1p. Conditional temperature-sensitive *ipl1*$^{ts-}$ mutant cells show abnormal ploidy, which suggests that the Ipl1p kinase activity somehow controls chromosome segregation. By contrast with yeast, mammals possess at least three members of this kinase family. The inactivation and the overexpression of two of these kinases leads to polyploidy (Bischoff et al., 1998; Tatsuka et al., 1998; Terada et al., 1998; Zhou et al., 1998). In addition, all three human kinases are overexpressed in tumour cells (Bischoff et al., 1998; Tatsuka et al., 1998; Zhou et al., 1998). One kinase (aurora2, AIK, BTAK, ARK1) localises to interphase and mitotic centrosomes and to the spindle poles. The inhibition of its activity leads to formation of a monopolar spindle, because its activity is necessary for centrosome separation (Glover et al., 1995; Roghi et al., 1998). Another kinase (Aik2, AIK2, aurora1) localises to the midbody; inhibition of its activity leads to formation of multinucleated cells, which indicates that the kinase is involved in cytokinesis (Tatsuka et al., 1998). A third kinase (aik3, AIE2) (Bernard et al., 1998) is localised in the centrosome only in anaphase (Kimura et al., 1999), and Tseng et al. (1998) show that its expression is testis specific.

Here, we present a comprehensive list of all the members of this new kinase family. Our aim is to show that a generic name is necessary for the family, to aid the understanding of the differences between paralogues in an organism and recognition of orthologues from different organisms. We propose the name Aurora/Ipl1p-related kinase (AIRK)*. In this paper, we also describe the conserved structure of these kinases and comment on the relationship between the subcellular localisation of these kinases and their function. Finally, we discuss how overexpression of these kinases could be involved in cellular transformation.

AURORA/IPL1P-RELATED KINASES IN DIFFERENT ORGANISMS

Table 1 lists all the known members of the AIRK family. Chan and Botstein (1993) identified Ipl1p during a screen designed to isolate *S. cerevisiae* mutants that show chromosome-segregation defects. The *Schizosaccharomyces pombe* genome sequencing programme identified an *S. pombe* AIRK.

*ARK which could have stood for 'Aurora-Related Kinase' is already used to designate 'actin regulating kinase'.

Table 1

Proposed nomenclature	Specie	Literature	Localisation	Gene name	GenBank Accession-number	References	Chromosome localisation
Ipl1	*S. cerevisiae*	Ipl1	ND		AL022245	Francisco et al., 1994	
SpAIRK	*S. pombe*		ND		U07163	*Unpublished (seq prog)*	
CeAIRK1	*C. elegans*	AIR-1	Centrosome		AF 071206	Schumacher et al., 1998a	
CeAIRK2		AIR-2	Chromosome and midbody		AF 071207	Schumacher et al., 1999a	
Aurora	*D. melanogaster*	Aurora	ND		X83465	Glover et al., 1995	
DmAIRK2		IAL	ND		AF121361	Reich et al., 1999	
XlAIRK1	*X. laevis*	pEg2	Centrosome		Z17207	Roghi et al., 1998	
MmAIRK1	*M. musculus*	ARK1 Ayk1 IAK	Centrosome		U69106	Shindo et al., 1998 Yanai et al., 1997 Gopalan et al., 1997	2
MmAIRK2		ARK2	Midbody	*Stk1*	U69107	Niwa et al., 1996 Shindo et al., 1998	11
MmAIRK3		AIE1			AF054620	Tseng et al., 1998	
RnAIRK2	*R. Norvegicus*	AIM1	Midbody		D89731	Terada et al., 1997	
HsAIRK1	*H. sapiens*	Aik BTAK ARK1 aurora2	Centrosome	*STK6* *[STK15]*	AF008551	Kimura et al., 1997a Kimura et al., 1997b Sen et al., 1997 Bischoff et al., 1998 Kimura et al., 1998 Shindo et al., 1998	20q13.2-q13.3
HsAIRK2		Aik2 ARK2 aurora1	Midbody	*STK12*	AF008552	Zhou et al., 1998 Bischoff et al., 1998 Prigent et al., 1998 Shindo et al., 1998	17p13
HsAIRK3		AIE2 Aik3	Anaphase-centrosome	*STK13*	AB017332	Tseng et al., 1998 Kimura et al., 1999 Bernard et al., 1998	19q13.3-ter
				STK6P		Kimura et al., 1997b	1q41-q42

The *Caenorhabditis elegans* genome encodes two AIRKs: AIR-1 and AIR-2. The AIR-1 kinase is required for the organisation of spindle but not for centrosome separation (Schumacher et al., 1998a). The AIR-2 kinase is involved in cytokinesis (Schumacher et al., 1998b).

Two genes that encode AIRKs have been found in *D. melanogaster* to date. Glover et al. (1995) identified the *aurora* gene during a search for mutants in which the centrosome cycle is affected. *Aurora* mutant embryos show monopolar spindles and pairs of unseparated centrosomes. A second AIRK (IAL) was found by the genome sequencing programme.

Paris et al. isolated the pEG_2 kinase by a differential screening in *Xenopus laevis* early development (Paris et al., 1988; Paris and Philippe, 1990). They identified two cDNAs that encode kinases that share a high degree of sequence identity, presumably because the *X. laevis* genome is tetraploid. pEG_2 is involved in centrosome separation and mitotic spindle assembly (Roghi et al., 1998). Recently, Andrésson and Ruderman (1998) have reported that pEG_2 kinase is one of the earliest components of the progesterone-induced oocyte-maturation pathway in *X. laevis*.

In *Mus musculus*, the *Stk1* gene (Niwa et al., 1996) encodes an AIRK (ARK2) that localises to the midbody (Shindo et al., 1998). A second gene (Shindo et al., 1998) encodes an AIRK that localises to the centrosome and the spindle pole. This has been given several names, such as ARK1 (Shindo et al., 1998), Ayk1 (Yanai et al., 1997) and IAK (Gopalan et al., 1997). A third AIRK (AIE1) is specifically expressed in testis (Tseng et al., 1998).

Only one AIRK, AIM1 (Aurora/IplIp-midbody-related kinase), has been isolated from *Rattus norvegicus* (Terada et al., 1998). The transfection of cells with an inactive mutant of the kinase leads to a cytokinesis defect.

Three different genes and one pseudogene (*STK6P*) have been found in *Homo sapiens*: (1) *STK6* (which seems to be identical to *STK15*) (Kimura et al., 1997b) encodes a centrosome-associated oncogenic kinase that was first named BTAK for breast-tumour-activated kinase (Sen et al., 1997) but which we term HsAIRK1. (2) *STK12* encodes a midbody-associated kinase HsAIRK2. (3) *STK13* encodes a testis-specific kinase (Tseng et al., 1998) that has been detected on centrosomes only during anaphase (Kimura et al., 1999). All three kinases are overexpressed in various types of tumour cell.

Because these kinases have been isolated simultaneously in different laboratories, they go by various names. Our proposed nomenclature divides the family into three groups on the basis

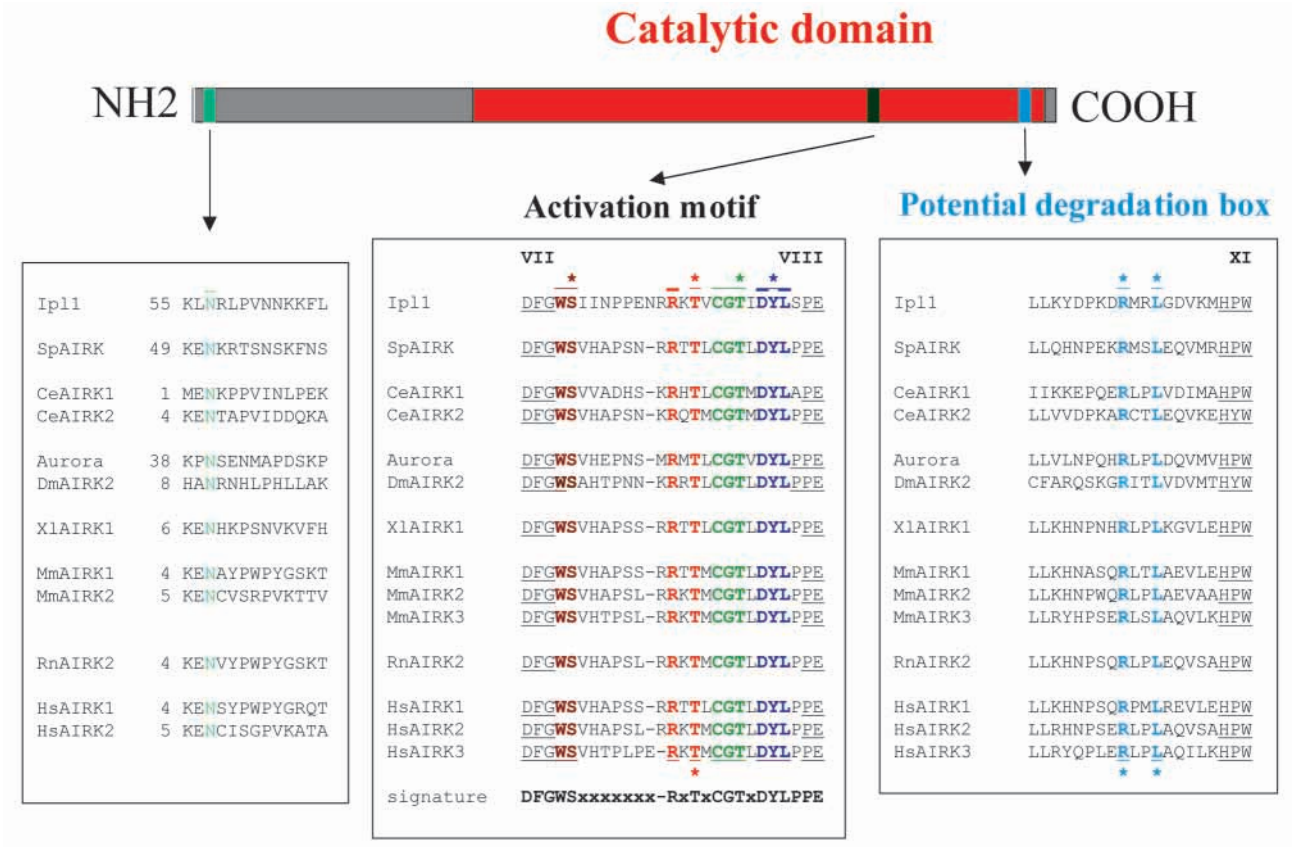

Fig. 1. The structure of Aurora/Ipl1p-related kinases (AIRKs). The C-terminal catalytic domain (red) and the N-terminal domain (grey). The most conserved sequence motif found in the N-terminal domain is shown in the left panel, it is not detected in MmARIK3 and HsAIRK3. Alignment of the aurora/Ipl1p-related kinase activation loop domain, between subdomains VII and VIII in the catalytic domain of these kinases is shown in the middle panel. Conserved amino-acids of subdomains VII and VIII are underlined in black. Coloured bold characters designate conserved amino-acids in the activation loop and coloured stars designate residues that potentially can be phosphorylated. The threonine residue equivalent of CDK1 T161, and a conserved arginine residue located one residue up-stream are shown in red. In black bold characters (bottom line) is the aurora/Ipl1p-related kinase signature. Sequences indicated in the right panel are potential degradation boxes, the conserved arginine and lysine residue of which are shown in blue. This potential degradation box (D box) is conserved in the C-terminal domain of all AIRKs.

of the kinases' localisation: AIRK1 kinases localise to the duplicated centrosomes during interphase and mitosis; AIRK2 kinases localise to the midbody; and AIRK3 kinases localise to the centrosome only in anaphase.

THE FUNCTIONS OF AIRKS

AIRK1 localises to the centrosome and the pole of the mitotic spindle. HsAIRK1 activity peaks in mitosis before that of CDK1 (Bischoff et al., 1998). Because AIRK1 is involved in centrosome separation, the inhibition of its kinase activity leads to spindle-bipolarity defects such as monopolar-spindle formation (Glover et al., 1995; Roghi et al., 1998); it is therefore indirectly involved in mitotic spindle assembly. During anaphase, AIRK2 localises to the mid-zone of the cell. During telophase, it is located in the telophase disc and within the post-mitotic bridge. By contrast with HsAIRK1 activity, HsAIRK2 activity peaks after that of CDK1 in mitosis (Bischoff et al., 1998). The kinase appears be involved in later mitotic events, and inhibition of AIRK2 leads to a cytokinesis defect (Terada et al., 1998).

The function of AIRK3 has not yet been elucidated, but its localisation at the centrosome, observed in anaphase only, suggests that it has an intermediate role after AIRK1 and before AIRK2 (Kimura et al., 1999). The functions of each of the three kinases are closely related to the dynamics of both the bipolar mitotic spindle and the microtubules of the midbody.

Evidence now suggests that AIRKs also play roles during meiosis. XlAIRK1, for instance, has been reported to be an early player in the cascade of reactions (presumably phosphorylation reactions) triggered by progesterone during oocyte maturation (Andrésson and Ruderman, 1998). The exact role of XlAIRK1 in maturation is still unclear, although the ectopic expression of the active kinase accelerates germinal-vesicle breakdown (Andrésson and Ruderman, 1998). Furthermore, in the case of *C. elegans*, CeAIRK2 (AIR-2) is necessary for normal meiotic division. Whenever antisense mRNA neutralises CeAIRK1 during early development, abnormal meiosis and polyploid embryos are the end result (Schumacher et al., 1998b); this implies that AIRKs are also involved in meiotic spindle dynamics.

AIRK SUBSTRATES

Kinesin-related proteins

At first, the presumptive role of AIRKs in centrosome separation, chromosome segregation and cytokinesis suggested that they play a part in microtubule-dependent mechanisms. Purified tubulins are not phosphorylated by XlAIRK1 in vitro (R. Giet and C. Prigent, unpublished results). Immunoprecipitation experiments performed with various AIRKs revealed that they have high molecular mass substrates (Kimura et al., 1998). Furthermore, we recently identified the KRP (kinesin-related protein) XlEg5 in XlAIRK1 immunoprecipitates (Giet et al., 1999a). The localisation of both proteins through indirect immunofluorescence in *X. laevis* cultured cells revealed that the kinase first localises to the duplicated centrosomes, and that, later on, XlEg5 starts to aggregate at a point precisely between the two centrosomes (Giet et al., 1999a). Both proteins then localise to centrosomes until the metaphase spindle assembles. The KRP and the kinase were previously reported to be involved in centrosome separation (Roghi et al., 1998; Blangy et al., 1995; Giet and Prigent, 1999b). In *S. cerevisiae*, genetic interaction between *CIN8* (which is homologous to XlEg5) and *IPL1* (which is homologous to XlAIRK1) was described in a search for genes that suppress Cin8p defects (Geiser et al., 1997). In *Drosophila*, mutations in the *aurora* and *KLP61F* genes lead to monopolar-spindle formation (Heck et al., 1993; Glover et al., 1995).

XlAIRK1 phosphorylates XlEg5 on a serine residue in the stalk domain that is involved in the dimerisation and tetramerisation of kinesin. Tetramerisation of HsEg5 is necessary for localisation of kinesin to the mitotic apparatus (Blangy et al., 1998). The role of phosphorylation of XlEg5 by XlAIRK1 has not yet been elucidated, but phosphorylation might regulate the oligomeric state of XlEg5. The CDK1 activity that peaks after AIRK1 (Bischoff et al., 1998) then regulates the binding of XlEg5 to spindle microtubules and to the dynactin-complex subunit $p150^{Glued}$ (Blangy et al., 1995, 1997).

The motor proteins that participate in microtubule dynamics, such as kinesins, kinesin-related proteins and dynein, are good candidates for substrates of AIRKs. All these motors move along microtubules, displacing cargo particles or other microtubules. The inhibition of motor-protein activities such as XCTK2 and Xklp2 leads to mitotic defects that are reminiscent of phenotypes obtained when AIRKs are inhibited (Sawin et al., 1992; Blangy et al., 1995; Boleti et al., 1996; Kashina et al., 1997; Walczak et al., 1997; Wittmann et al., 1998; Blangy et al., 1998).

Kinetochore proteins

Glc7p is the *S. cerevisiae* catalytic subunit of a type I phosphatase that acts in opposition to Ipl1p. Overexpression of Glc7p results in chromosome missegregation, whereas mutation of *GLC7* partially suppresses the Ipl1 phenotype (Francisco et al., 1994). When the type I phosphatase was overexpressed, the increased level of PP1, which appears during anaphase B accelerated the mitosis exit phase (Fernandez et al., 1992). Such a premature exit from mitosis might result in chromosome missegregation. Might exit from mitosis be regulated by the balance between AIRK and type 1 phosphatase activities? Such a hypothesis is consistent with a recent report suggesting that entry into and exit from mitosis are both regulated by type 1 phosphatases (Tournebize et al., 1997).

Francisco et al. (1994) have suggested that the phosphorylation/dephosphorylation reactions controlled by Ipl1p and Glc7p regulate the activity of identical substrate(s) in a phosphorylation cascade that controls chromosome segregation. This phenomenon was nicely demonstrated recently: Biggins et al. (1999) and Sassoon et al. (1999) showed that Ndc10p, a component of the kinetochore, is a substrate of both the Ipl1p kinase and the Glc7p phosphatase. The phosphorylation state of Ndc10p has no influence on its DNA-binding activity; however, dephosphorylation of Ndc10p by the type 1 phosphatase Glc7p is necessary for capture of the chromosome kinetochores by microtubules (Sassoon et al., 1999), whereas phosphorylation of Ndc10p by Ipl1p disrupts

the binding process (Biggins et al., 1999). Exit from mitosis is controlled by the spindle/kinetochore checkpoint. Clearly, the identification of the kinetochore protein Ndc10p as a common substrate for Ipl1p and Glc7p provides evidence of a role for AIRKs in the establishment of the mitotic checkpoint. Additionally HsAIRK1 associates with Cdc20, which activates the anaphase-promoting complex (APC) (Farruggio et al., 1999).

The role of type 1 phosphatase in the dephosphorylation of motor proteins has not yet been investigated. The human AIRK that is involved in kinetochore protein phosphorylation remains to be pinpointed.

STRUCTURE AND REGULATION OF AIRKs

Previous research has demonstrated that AIRK mRNA and protein levels reach their peak in the G_2/M phases of the cell cycle (Niwa et al., 1996; Kimura et al., 1997a; Gopalan et al., 1997; Shindo et al., 1998; Terada et al., 1998; Bischoff et al., 1998) and, interestingly enough, the activities of AIRKS also appear to be cell cycle regulated. The HsAIRK1 and HsAIRK2 activities reach their peak during mitosis, but HsAIRK1 activity is maximal before that of CDK1 whereas HsAIRK2 activity reaches a maximum after that of CDK1, suggesting a very precise timing of activation (Bischoff et al., 1998).

HsAIRK1 is also more active when immunoprecipitated from M phase cells than from S phase cells, which suggests that the kinase undergoes an activating post-translational modification upon entry into mitosis (Kimura et al., 1997a). The same is true for MmAIRK1 (Gopalan et al., 1997) and XlAIRK1 (Y. Arlot-Bonnemains, R. Giet and C. Prigent, unpublished). Furthermore, prior to this study, we demonstrated that the activity of a XlAIRK1 expressed in *E. coli* is stimulated upon incubation in *X. laevis* egg extract and that activation is concomitant with phosphorylation of the protein (Giet et al., 1999b).

All AIRKs share a common protein kinase structure (Fig. 1, top). They have a catalytic domain that is highly conserved within the family, and comprises a very short C-terminal domain and an N-terminal domain that varies in size.

Most proteins belonging to a defined kinase family possess an identifiable amino acid signature within the catalytic domain, e.g. CDKs have a PSTAIRE motif (MacNeill and Nurse, 1993), and MAP kinases have a TEY motif (Zhang et al., 1995). Do AIRKs have a signature? The most conserved motif is found in the potential activation loop. The highest scores in a Blast search (Altschul et al., 1997) based on the XlAIRK1 sequence between subdomains VII and VIII (FGWSVHAPSSRRTTLCGTLDYLPPE) were from AIRKs; cyclic-AMP-dependent protein kinases (PKAs) were the next most similar. We suggest that the consensus sequence DFGWSxxxxxxxRxTxCGTxDYLPPE is a signature for AIRKs (Fig. 1, middle panel). Numerous protein kinases are activated by phosphorylation in this sequence. For instance, the T_{161} in the catalytic domain of CDK1, which is located in the activation domain between subdomain VII and subdomain VIII (Hanks and Quinn, 1991), is phosphorylated by CAK (CDK1-activating kinase), and its phosphorylation results in the activation of CDK1 (Fesquet et al., 1993; Poon et al., 1993).

Do AIRKs, therefore, possess a conserved phosphorylation site in their activation domains? The above consensus sequence contains a conserved threonine residue (Fig. 1, red asterisk). Moreover, the sequence around this residue is highly conserved. This RXT sequence is similar to that of the phosphorylation site within the activation domain of PKA. Mutation of the threonine residue in the RXT sequence of HsAIRK1 to an acidic residue, which mimics a constitutive phosphorylation, generates a hyperactive kinase (Bischoff et al., 1998). This indicates that the threonine residue is the target for an activating kinase.

The degradation of AIRKs at the end of mitosis might provide another level of regulation. The levels of these protein kinases are very low in G_1 phase, which suggests that the kinases are specifically degraded upon exit from mitosis (Terada et al., 1998; Kimura et al., 1997a). Classically, a conserved amino acid sequence named the D-box (for 'degradation box'), targets proteins such as cyclin B for degradation by the proteasome via the ubiquitin-dependent pathway upon exit from metaphase (Glotzer et al., 1991). Do AIRKs contain such a D-box? Alignment of subdomains X and XI of the catalytic domains of AIRKS shows that the two residues, an arginine and a leucine, that characterise the D-box are invariably conserved (Fig. 1, right panel). Until now, there has been no direct evidence for degradation of AIRKs by a ubiquitin-dependent or -independent pathway. Obviously, mutagenesis of arginine and/or leucine must be performed if we are to establish whether this is indeed a D-box.

In contrast to the catalytic domains, the N-terminal domains of AIRKs do not share any obvious sequence similarity. The most conserved motif that can be found is located at the very beginning of the N-terminal domain (Fig. 1, left panel). The length of this N-terminal domain varies from 7 residues (HsAIRK3) to 162 residues (aurora) (Fig. 2). The N-terminal domain seems to be responsible for the specificity of each kinase. So far, it has not been possible to rescue the temperature-sensitive *Ipl1*$^{ts-}$ mutant cells with various mammalian AIRKs. Moreover, the expression of the mammalian kinases accentuates the phenotype of *ipl1* cells, which suggests that they behave as dominant negative forms of the budding yeast kinase (Kimura et al., 1997a; Gopalan et al., 1997; Bischoff et al., 1998). Expression of chimeric proteins in which the N-terminal domain of Ipl1p was fused to the catalytic domain of HsAIRK1 (Bischoff et al., 1998) partially rescues the phenotype of *ipl1*$^{ts-}$ cells. This suggests that the role of the N-terminal domain is species-specific.

What is the role of this N-terminal domain? Given the above findings, this domain probably targets the kinase either for subcellular localisation or for substrate recognition. As for several other protein kinases, the N-terminal domain might also regulate the kinase activity of the protein (Colbran et al., 1989). All these hypotheses need to be tested.

The presence of only one AIRK in yeast suggests that the kinase is the ancestor of the three mammalian kinases. Among the three human and mouse paralogues, only the catalytic domain is highly conserved. The various kinases have thus evolved from an ancestral kinase by inheriting different N-terminal domains, which are not very well conserved even among orthologues, suggesting species-specificity of the domain. Because only the chimera that contains the yeast Ipl1p N-terminal domain can partially rescue the phenotype of *ipl1*$^{ts-}$

Fig. 2. The N-terminal domain sequence of Aurora/Ipl1p-related kinases. We considered the N-terminal domain to be the sequence that ended eight residues upstream from the catalytic sequence GKGK. The length of the domain is indicated on the right of figure.

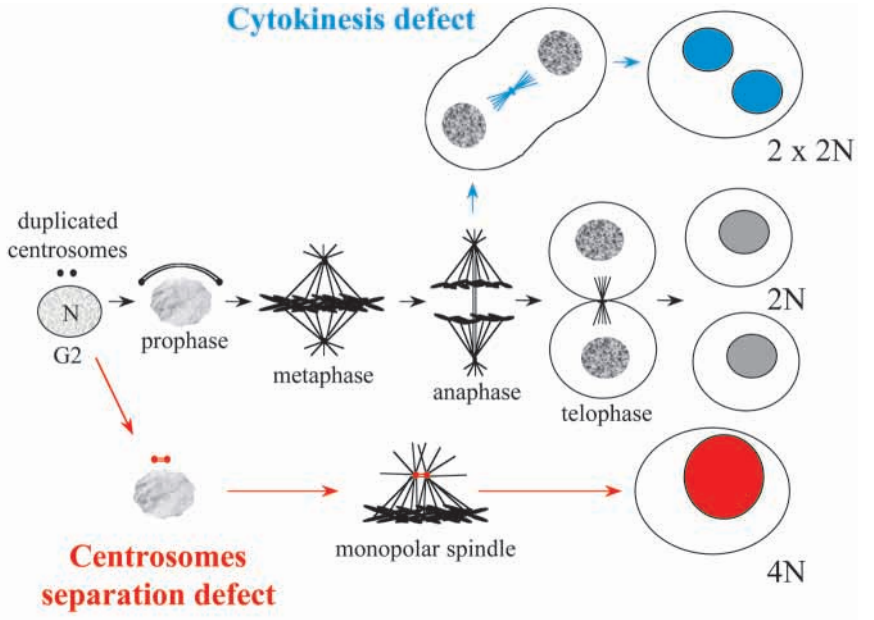

Fig. 3. The polyploidy mechanism generated by centrosome-separation defects and cytokinesis defects. The presence of unseparated centrosomes (red) leads to monopolar spindle formation, such spindles are unable to ensure chromosome segregation (in this diagram, kinetochores are captured by microtubules). A cytokinesis defect (blue) obviously leads to formation of polynucleated cells.

cells, each of the human AIRKs might have inherited only part of the function of the yeast kinase.

AIRKS AND CANCER

HsAIRK1, HsAIRK2 and HsAIRK3 are overexpressed in various tumour cells (Bischoff et al., 1998; Zhou et al., 1998; Tatsuka et al., 1998; Tanaka et al., 1999). HsAIRK3 maps to chromosome 19q13.3-ter (Bernard et al., 1998; Kimura et al., 1999). Translocation, deletion and amplification of this region have been reported in various cancer tissues (Bicher et al., 1997; Hoglund et al., 1998). Elevated levels of HsAIRK3 expression have been observed only in a restricted number of cancer cells, however (Kimura et al., 1999). A relationship between tumorigenesis and HsAIRK3 expression therefore has yet to be clearly demonstrated.

The HsAIRK2 gene maps to chromosome 17p13 (Prigent et al., 1998; Katayama et al., 1998). This region is deleted in various human cancers (Eiriksdottir et al., 1998), but high levels of the expression of HsAIRK2 are evident in cell lines derived from colorectal tumours that show abnormal ploidy (Tatsuka et al., 1998). The ectopic overexpression of HsAIRK2 in cultured cells produces multinuclear cells, in which nuclei sometimes fuse to yield cells with large nuclei.

The HsAIRK1 gene maps to chromosome 20q13 (Shindo et al., 1998). This region is amplified in several human cancers (Kallioniemi et al., 1994; Schlegel et al., 1995); in particular, the amplification of the 20q13 region has been associated with poor prognosis (Isola et al., 1995). Furthermore, HsAIRK1 is overexpressed in various cancer cells (breast, ovarian, colon, prostate, neuroblastoma and cervical cancer cell lines). The overexpression observed was due not only to gene amplification but sometimes to the

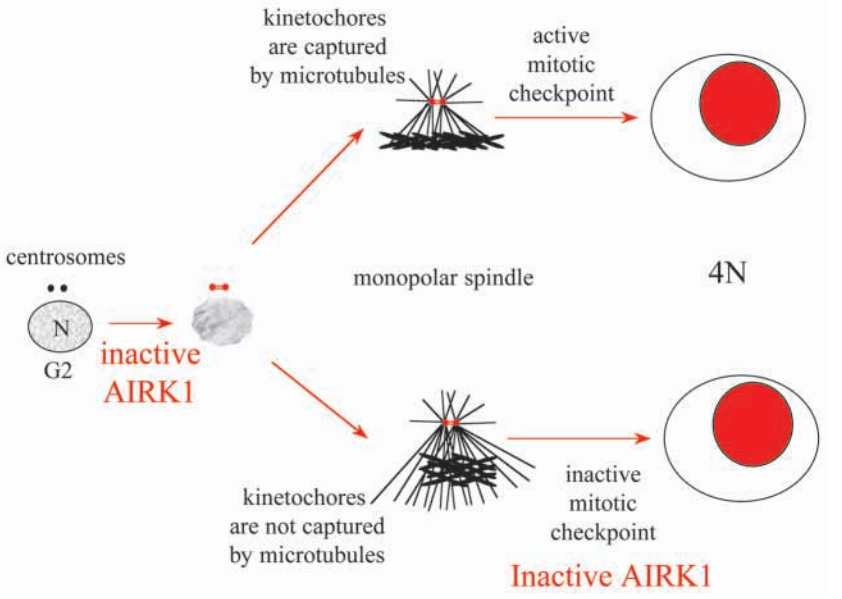

Fig. 4. Is ARK1 involved in the mitotic checkpoint? If kinetochores are captured by microtubules, the mitotic checkpoint does not sense the monopolar-spindle defect, and cytokinesis leads to polyploidy. If kinetochores are not captured, then the inactivation of the mitotic checkpoint is necessary to allow the cells to become polyploid. ARK1 would be involved in this checkpoint.

deregulation of mRNA expression without gene amplification (Bischoff et al., 1998). This observation indicates that there is a strong correlation between a gain in HsAIRK1 kinase activity and cancer. The ectopic expression of the kinase in rat cells and human cells eventually demonstrated that HsAIRK1 possesses a potential oncogenic activity because it produces a transformed phenotype (Bischoff et al., 1998; Zhou et al., 1998).

This transformation is associated with the abnormal centrosome number (amplification) that is currently associated with genomic instability and cancer (Weber et al., 1998; Xu et al., 1999). Cells that contain more than two centrosomes cannot assemble bipolar spindles. Consequently, mitosis brings about chromosome segregation defects, and thus aneuploidy.

POLYPLOIDY

Defects in centrosome separation lead to the formation of monopolar spindles, which are not functional: chromosome segregation cannot be ensured; cytokinesis does not occur; and cells re-enter G_1 phase without division (Fig. 3). The effect of a cytokinesis defect is even more obvious. The chromosomes are segregated, but the cells do not divide; the cells therefore become multinucleated (Fig. 3). The nuclei eventually fuse, and the cells become polyploid.

Overexpression of active AIRK1 or AIRK2 leads to polyploidy as does the overexpression of the inactive AIRK1 or AIRK2 (Bischoff et al., 1998; Zhou et al., 1998; Tatsuka et al., 1998). How can the overexpression both of active and of inactive proteins lead to the same phenotype? The increase in ploidy in *S. cerevisiae ipl1* mutant cells is due to production of an inactivated kinase, which indicates that the polyploid phenotype is not a consequence of any increase in kinase activity (Francisco et al., 1994; Glover et al., 1995).

In mammalian cells, one possible explanation is that the overexpressed active kinase behaves as a dominant negative mutant that inhibits other AIRKs. For instance, overexpressed AIRK1 might act as a dominant negative form of AIRK2 and thus inhibit cytokinesis. This phenomenon has been demonstrated in budding yeast, in which overexpression of the human kinase accentuated the phenoptype of $ipl1^{ts-}$ cells. Given that the Ipl1p N-terminal domain fused to the HsAIRK1 catalytic domain partially complements $Ipl1^{ts-}$ mutant cells (Bischoff et al., 1998), the presence of a different N-terminal domain seems to be sufficient for a dominant negative effect.

Another set of striking results relates to the behaviour of cells in which HsAIRK1 has been overexpressed. When active kinase is overexpressed, the polyploïd cells eventually become immortalised, whereas when inactive kinase is overexpressed, cells eventually die (Tatsuka et al., 1998). These results suggest that AIRKs are linked to an apoptosis pathway and to the mitotic checkpoint.

CHECKPOINT

Cells possess various control mechanisms that monitor mitosis, for instance, the depolymerisation of spindle microtubules by nocodazole triggers mitotic arrest because the cell has sensed that chromosome kinetochores have not captured the microtubules. The cell does not trigger exit from mitosis until the last chromosome has been attached to the microtubule and aligned at the metaphase plate (Rieder et al., 1994, 1995). Strikingly, if AIRK1 activity is inhibited, although chromosome segregation does not occur, the cell seems to overcome this defect, proceeding into the next G_1 phase and starting another cell cycle. There are two possible interpretations of this finding. First, chromosome kinetochores might capture microtubules, but, because the spindle is not functional, segregation does not occur. In this case, the spindle checkpoint remains inactivated because it does not sense spindle defects (Sluder et al., 1997), and cell cycle progression leads to formation of polyploid cells (Fig. 4). Second, chromosome kinetochores might not capture microtubules, because the spindle is not functional (monopolarity); consequently, the chromosomes cannot be separated. In the latter alternative, the fact that the cell is not arrested in mitosis suggests that AIRK1 is involved in the mitotic checkpoint (Fig. 4).

In *S. cerevisiae*, in which Ipl1p activity is counteracted by type 1 phosphatase Glc7p (Francisco et al., 1995), loss of Glc7p function activates the spindle/kinetochore checkpoint (Bloecher and Tatchell, 1999). Only checkpoint deficiencies then allow abnormal mitosis in which there are defects in chromosome segregation to proceed. If HsAIRK1 activity is also counteracted by a type 1 phosphatase, it is tempting to think that the overexpression of HsAIRK1 in human cells deregulates the balance between the kinase and the phosphatase and mimics the $GLC7^{-}$ phenotype. In the presence of a high level of HsAIRK1 activity, (1) kinetochore-microtubule bonds are destabilised because kinetochore proteins such as Ndcd10p cannot remain in a stable dephosphorylated state to ensure the binding of the microtubules to the kinetochores (Biggins et al., 1999; Sassoon et al., 1999); (2) somehow the efficiency of the spindle/kinetochore checkpoint is lowered and the PP1 cannot counteract the high level of AIRK1 activity (Bloecher and Tatchell, 1999), allowing genetic instability that can give rise to cancer cells (Hartwell, 1992). The finding in yeast that a kinetochore protein is a common substrate for a type 1 phosphatase and an AIRK provides a strong argument for such a mechanism. One implication is that AIRK1 activity participates in the establishment/maintenance of the mitotic checkpoint. In this hypothesis, AIRK1 should have at least two distinct functions: (1) in the assembly and/or the stability of the spindle; (2) in control of exit from mitosis, because it is a component of the mitotic checkpoint (Fig. 4). A dual function would not be surprising, because the same overall mechanism has already been described for other checkpoints: the proteins involved in DNA repair, for instance, are also involved in the cell cycle arrest at checkpoints after DNA damage has occurred (Paulovich et al., 1997).

Various mechanisms that lead to polyploidy or aneuploidy are known. Two of them, mitotic-checkpoint override and cleavage-furrow inhibition, might correspond to phenotypes observed after overexpression and inhibition of AIRK1 and AIRK2 has taken place (Andréassen et al., 1996). AIRK1 is a centrosome protein. Several tumour suppressors have been localised to mitotic centrosomes and directly participate in centrosome cycles (Minn et al., 1996; Hsu and White, 1998). For instance, inhibition of p53 triggers abnormal centrosome amplification (Fukasawa et al., 1996, 1997), which is reminiscent of the phenotypes obtained after overexpression of HsAIRK1 (Zhou et al., 1998). The machinery responsible for

the mitotic checkpoint has a very restricted localisation within the cell and is present solely around the spindle (Rieder et al., 1997). Centrosomes are now central to numerous biological problems such as cell cycle control, checkpoints, genomic stability, polypoidy and cancer (Hartwell, 1992; Hartwell and Kastan, 1994; Winey, 1996, for review).

CONCLUSION

The AIRKs represent a new, large family of mitotic kinases, the functions of which are closely linked to the bipolar microtubule spindle dynamic present both at mitosis and meiosis. An HsAIRK is overexpressed in various human cancers and possesses a oncogenic activity (Bishoff et al., 1998; Zhou et al., 1998). These kinases are therefore potential targets for anti-tumour drugs. Could inhibition of HsAIRK1 activity in cancer cells in which the kinase is overexpressed reverse the cancer phenotype? This is an attractive hypothesis, and many research programs are screening for kinases inhibitors of AIRKs, especially of HsAIRK1.

Meanwhile, two major points remain to be addressed. (1) What event(s) triggers *HsARK1* gene amplification in a normal cell? (2) What is the exact role of the different AIRKs during mitosis? The function of HsARK1 is beginning to emerge: the kinase is involved in both centrosome separation and bipolar spindle assembly, presumably because it regulates microtubule-driving motor proteins such as Eg5 (Glover et al., 1995; Roghi et al., 1998; Giet et al., 1999b). The functions of the other kinases remain to be pinpointed. Although a loss in HsAIRK2 activity inhibits cytokinesis, the mechanism at the molecular level is unknown, and there is even less information on the function of HsAIRK3.

AIRK1 also clearly belongs to a cascade of kinases that remains to be identify. Phosphorylation of the threonine residue between subdomain VII and VIII in the catalytic domain of HsAIRK1 clearly activates the kinase (Bishoff et al., 1998). Furthermore, XlAIRK1 is activated upon progesterone activation of the *X. laevis* oocyte (Andrésson and Ruderman, 1998). *Xenopus* oocyte maturation is a powerful biochemical system that will be helpful for identifying the AIRK1-activating kinase and the AIRK1 substrates.

Because AIRKs kinases appear to control the ploidy state of eukaryotic cells, their activities must somehow be involved in the mitotic checkpoint. The discovery that Ndc10p is one of the budding yeast AIRK substrates was a breakthrough (Biggins et al., 1999; Sassoon et al., 1999). Ndc10p is involved in the capture of microtubules by kinetochores, which is the mitotic event sensed by the mitotic checkpoint (Rieder et al., 1994). But, because the yeast genome encodes only one AIRK, the mammalian AIRK involved in this mechanism remains to be identified.

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