A homologue of *Drosophila aurora* kinase is oncogenic and amplified in human colorectal cancers

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Genetic and biochemical studies in lower eukaryotes have identified several proteins that ensure accurate segregation of chromosomes. These include the *Drosophila aurora* and yeast Ipl1 kinases that are required for centrosome maturation and chromosome segregation. We have identified two human homologues of these genes, termed *aurora1* and *aurora2*, that encode cell-cycle-regulated serine/threonine kinases. Here we demonstrate that the *aurora2* gene maps to chromosome 20q13, a region amplified in a variety of human cancers, including a significant number of colorectal malignancies. We propose that *aurora2* may be a target of this amplicon since its DNA is amplified and its RNA overexpressed, in more than 50% of primary colorectal cancers. Furthermore, overexpression of *aurora2* transforms rodent fibroblasts. These observations implicate *aurora2* as a potential oncogene in many colon, breast and other solid tumors, and identify centrosome-associated proteins as novel targets for cancer therapy.

Keywords: 20q13 amplicon/centrosome/colon cancer/ oncogene/serine-threonine kinase

Introduction

Chromosomal abnormalities are a hallmark of human cancer, reflecting the deleterious consequences of the gain or loss of genetic information (Boveri, 1929; Hartwell and Kastan, 1994; Mitelman *et al.*, 1997). Some of these defects may have a causal role in cellular transformation due to loss of a negative growth regulator or a gene responsible for maintenance of genome integrity, or through the amplification, overexpression or mutational activation of an oncogene (Hunter, 1997; Kinzler and Vogelstein, 1997). Alternatively, these abnormalities may be a consequence of tumor progression, where disruption of mitotic checkpoints can result in abnormal nuclei, missegregated chromosomes and aneuploidy (Elledge, 1996; Sherr, 1996).

A direct connection between the cell cycle and cancer was first established with the observation that the cyclin D1 gene was amplified in a subset of human cancers (Motokura *et al.*, 1991; Hunter and Pines, 1994). The subsequent discovery that the tumor suppressor p53 regulates p21, an inhibitor of cyclin-dependent kinases (el-Deiry *et al.*, 1993; Xiong *et al.*, 1993), as well as the identification of p16—another cyclin-dependent kinase inhibitor—as a major tumor suppressor gene (Elledge, 1996; Sherr, 1996), has firmly entrenched the view that misregulation of the cell cycle machinery can have enormous impact on cellular proliferation. Based on the prevalence of genetic abnormalities in human cancer, it is plausible that proteins involved in maintaining the integrity of chromosome segregation may also play a role, directly or indirectly, in cellular transformation. The fidelity of chromosome segregation is monitored by mitotic checkpoints that delay entry into mitosis until a functional centrosome is present, or delay progression beyond anaphase until the chromosomes are aligned on the metaphase plate by the mitotic spindle. In normal cells, centrosomes play an important role in coordinating the changes required for the onset of mitosis, serving as an anchor for reorganization of the cytoplasmic microtubules into a mitotic spindle apparatus and for recruitment of numerous structural, motor and catalytic proteins to the centrosome complex. Proper execution of this process ensures that each daughter cell receives the correct number of chromosomes. Recent studies suggest that a G₂/M checkpoint may exist to ensure the integrity of this process of centrosome maturation (Nigg *et al.*, 1996).

Genetic and biochemical studies in yeast and *Drosophila* have identified several proteins involved in chromosome segregation and spindle assembly. Disruption of these proteins results in chromosome missegregation, monopolar or disrupted spindles and/or abnormal nuclei. Several of these proteins represent distinct families of protein serine/threonine kinases, including: Cdc2, a cyclin-dependent kinase conserved from yeast to mammals that is required for centrosome separation and formation of a bipolar spindle (Sherr, 1994, 1996; Morgan, 1995; Elledge, 1996); Mps1, a *Saccharomyces cerevisiae* dual specificity kinase required for spindle pole body duplication (Weiss and Winey, 1996); Bub1, a *S.cerevisiae* and mammalian mitotic checkpoint kinase (Hoyt *et al.*, 1991; Taylor and McKeon, 1997); PLK1, a mammalian homologue of polo, Cdc5p and plol kinases from *Drosophila*, budding and fission yeast, respectively, that communicates the presence of a functional centrosome to the Cdk/cyclin complex prior to entry into mitosis (Lane and Nigg, 1996, 1997); and the Ipl1 and *aurora* kinases from *S.cerevisiae* and *Drosophila*, respectively, that are required for centrosome

A

B

Fig. 1. Homology between human, *Xenopus*, *Drosophila* and yeast auroras. (A) The sequences for human aurora1 and aurora2 were deduced from full-length cDNA clones isolated from normal duodenum, pancreatic carcinoma and primary colorectal carcinoma libraries. *Xenopus* p46B (PIR:S53343), *Drosophila* aurora (PIR:A56220) and *S.cerevisiae* Ipl1 (SWISS-PROT:P38991) are included in the alignment. The alignment was generated by also including the two murine (DDBJ/EMBL/GenBank accession Nos D21099 and GB:U80932), an additional *Xenopus* (PIR:S53342) and two *C.elegans* (DDBJ/EMBL/GenBank accession Nos U53336 and U97196) sequences as input into msa, a parallel-coded multiple sequence alignment program that was run on MasPar MP2216 supercomputer. Boxed residues are common to three or more of the sequences; shaded residues represent regions of amino acid similarity between two or more sequences; overlines correspond to the conserved Aurora Box1 and Aurora Box2 sequences; the arrow denotes the start of the C-terminal serine/threonine kinase domain; the circled residue indicates the location of a single nucleotide polymorphism described in the text; solid circles correspond to the location of various yeast and *Drosophila* mutants (Francisco *et al.*, 1994; Glover *et al.*, 1995); and stars denote the site of the kinase-inactivating and -activating point mutants described in the text. (B) Schematic domain structure of human aurora1 and aurora2.

separation and chromosome segregation (Francisco *et al.*, 1994; Glover *et al.*, 1995). Among these kinases, only PLK1 has been shown to be transforming (Smith *et al.*, 1997), although many are implicated to play a role in

the genotypic changes associated with immortalized cells, possibly due to the presence of a compromised checkpoint (Hoyt *et al.*, 1991; Lane and Nigg, 1996, 1997; Taylor and McKeon, 1997).

Here, we describe the identification and characterization of two human homologues of *Drosophila* aurora and yeast Ipl1, that we have named aurora1 and aurora2.

Results

Structural comparison of aurora homologues

We initiated a PCR-based screen in order to identify novel colon cancer-associated kinases. One of these clones encoded a protein with homology to the aurora protein kinase from *Drosophila melanogaster* and the Ipl1 kinase from *S.cerevisiae* (Francisco *et al.*, 1994; Glover *et al.*, 1995). While using this fragment to screen for a full-length cDNA clone, we also identified a weakly hybridizing clone that was found to encode a related kinase. We refer to these genes as *aurora1* and *aurora2*, to reflect their homology to each other and to the *Drosophila* aurora

kinase. The *aurora1* cDNA contained a 1032 bp open reading frame that encodes a 344 amino acid polypeptide with a predicted molecular mass of 39.3 kDa. The *aurora2* cDNA contained a 1209 bp open reading frame that encodes a 403 amino acid polypeptide with a predicted molecular mass of 45.8 kDa. Two additional human *aurora* pseudogenes were also identified as expressed transcripts that are each contained on single exons and maintain striking DNA homology to either *aurora1* or 2, yet exhibit multiple frame shifts (G.D.Plowman, unpublished). During preparation of this manuscript, a partial sequence of *BTAK* (Sen *et al.*, 1997), a breast tumor-associated kinase, was reported that appears to be a fragment of human *aurora2*. A second paper reported the sequence of human *aik* (Kimura *et al.*, 1997), a cell cycle-regulated protein localized to spindle pole bodies. The published sequence shares 92% amino acid identity with our sequence of

Fig. 2. The catalytic domain of aurora2, but not aurora1, partially complements the yeast *ipl1-1* mutation. (A) Map of various yeast transformants of strain CCY464-1D streaked onto SC-URA plates. Clockwise from top: vector, empty expression vector; Ipl1/A1KM, N-terminal domain of Ipl1 fused with the C-terminal portion of a kinase-dead *aurora1* construct; Ipl1/A1, N-terminal domain of Ipl1 fused with the C-terminal portion of wild-type *aurora1*; Ipl1/A2, N-terminal domain of Ipl1 fused with the C-terminal portion of wild-type *aurora2*; Ipl1/A2KM, N-terminal domain of Ipl1 fused with the C-terminal portion of a kinase-dead *aurora2* construct; and Ipl1, wild-type Ipl1. (B) Plate grown at the permissive temperature of 26°C. (C) Plate grown at the restrictive temperature of 34°C. (D) Plate grown at the restrictive temperature of 37°C.

Fig. 3. Aurora1 and aurora2 proteins are cell cycle-regulated and localized to mitotic structures. Exponentially growing HeLa cells were synchronized at the G₁/S transition by a double thymidine/aphidicolin block. Separate plates (10 cm) were harvested for FACS analysis, RNA isolation, protein quantitation and kinase assays at the indicated times. (A) FACS analysis was performed on exponentially growing HeLa cells, as well as cells harvested at 0, 4, 9, 10 and 12 h after release. (B) Northern blots of synchronized HeLa cells probed with a ³²P-labeled *aurora1* cDNA (top panel), a ³²P-labeled *aurora2* cDNA (middle panel), and a ³²P-labeled actin cDNA (bottom panel). Equal amounts of total RNA (10 µg) were loaded in each lane. (C) Immunoblots probed with protein A-purified anti-aurora1 antibodies (top panel), anti-aurora2 antibodies (middle panel) or anti-p34^{cdc2} antibodies (bottom panel). Equal amounts of total cellular protein (50 µg) were loaded in each lane. (D) *In vitro* kinase assays with anti-aurora1 immune complexes (top panel) using GST2TK (PKA phosphorylation site) as a substrate, with anti-aurora2 immune complexes (middle panel) using myelin basic protein (MBP) as an artificial substrate, or anti-p34^{cdc2} immune complexes (bottom panel) using histone H1 as a substrate. Equal amounts of total HeLa cell protein (500 µg) were used for each immunoprecipitation. (E) Aurora1 is localized to the midzone and post-mitotic bridge. HeLa cells at various stages of mitosis were stained for DNA, α-tubulin and aurora1. Top panels, DAPI staining of DNA; middle panels, α-tubulin immunostaining; bottom panel, aurora1 immunostaining. (F) Aurora2 is localized to the mitotic spindle of metaphase and anaphase cells. HeLa cells at various stages of mitosis were stained for DNA, α-tubulin and aurora2. Top panels, DAPI staining of DNA; middle panels, α-tubulin immunostaining; bottom panel, aurora2 immunostaining.

A

B

RNA

C

protein

D

kinase

E

F

human *aurora2*. We believe that *aurora2* and *aik* are identical and six frameshifts resulting from sequencing errors explain the small differences in the published sequence. Three additional papers provide the sequence of *AYK1* (Yanai *et al.*, 1997), a meiotic-regulated gene and *IAK1* (Gopalan *et al.*, 1997), both of which appear to be the murine orthologues of *aurora2*, and *AIM-1* (Terada *et al.*, 1998) which is a rat orthologue of *aurora1*. The current report describes the first complete sequence for both human *aurora1* and *aurora2*.

The deduced amino acid sequences of human *aurora1* and *aurora2* are presented in Figure 1A, aligned with the yeast and *Drosophila* homologues *Ipl1* and *aurora* and an additional homologue p46B from *Xenopus laevis*. Human *aurora2* protein shares 57%, 63%, 43% and 41% identity over its entire length with human *aurora1*, *Xenopus* p46B, *Drosophila* *aurora* and *Ipl1*, respectively. The four sequences contain a C-terminal domain with all the subdomains characteristic of a serine/threonine kinase. The kinase domain of human *aurora2* shares 74%, 62% and 49% amino acid identity with human *aurora1*, *Drosophila* *aurora* and *Ipl1*, respectively, and 83.5% identity with two amphibian homologues present in *Xenopus* [p46A (p46Eg22, PIR:S53342) and p46B (p46Eg265, PIR:S53343)]. *Drosophila* *aurora* is most related to human *aurora1* whereas yeast *Ipl1* is most related to *aurora2*. Whereas a single *aurora*-like kinase is present in yeast, at least two members are present in *Caenorhabditis elegans* (DDBJ/EMBL/GenBank accession No. U53336, gene K07C11.2 and U97196, gene B0207.4). The deduced catalytic domains of these *C.elegans* proteins share 55% and 64% amino acid sequence identity to the human *aurora2* kinase domain. We predict that an additional *aurora* homologue will ultimately be identified in *Drosophila* as characterization of its genome nears completion.

The 129 and 73 amino acid N-terminal domains of human *aurora2* and *aurora1* share limited homology with each other and with the analogous 160 and 100 amino acid domains of *Drosophila* *aurora* and yeast *Ipl1*. However, the N-terminal regions of human and mouse *aurora2* share 54% identity to each other and 28–30% identity to the two *Xenopus* proteins and together help define two distantly conserved motifs present in the non-catalytic region of all auroras (Figure 1A and B). These motifs are composed predominantly of conserved hydrophobic and polar residues. The first motif spans 18–37 amino acids (Aurora Box1), with *aurora1* and yeast *Ipl1* lacking the central portion and the second motif spans 21 amino acids (Aurora Box 2; see overlines in Figure 1A). Several potential serine and threonine phosphorylation sites are also conserved among these proteins, including a protein kinase A phosphorylation motif RRXT in the activation loop of the kinase. A temperature-sensitive mutant of the yeast *Ipl1* gene has a threonine to alanine substitution at this site (Francisco *et al.*, 1994), suggesting that phosphorylation on this threonine residue within the activation loop may be biologically relevant. Additional mutations in the yeast (Francisco *et al.*, 1994) and *Drosophila* (Glover *et al.*, 1995) homologues of *aurora* have been mapped exclusively to the kinase domain, except for a single *Drosophila* mutant (Glover *et al.*, 1995) that changes an aspartate to an alanine at residue 47 within the N-terminal Aurora Box1. Since these mutations result in abnormal nuclei,

chromosome missegregation and monopolar spindles, these findings suggest that the catalytic activity of the auroras may play an important role in centrosome biology.

Aurora2 partially complements *Ipl1*

To determine whether human *aurora1* and/or *aurora2* are functionally equivalent to their *S.cerevisiae* homologue *Ipl1*, we attempted to complement the *ipl1-1* temperature-sensitive mutant strain, CCY464-1D (Francisco *et al.*, 1994) with expression plasmids encoding the *aurora* proteins. The CCY464-1D strain is inviable above 34°C due to a mutation in *Ipl1* (Francisco *et al.*, 1994). Neither *aurora1* nor *aurora2* was able to complement the *ipl1-1* mutation at 37°C, probably due to an inhibition of cell growth on overexpression of the unique N-terminal domains of these proteins (unpublished observation). To circumvent this problem, we generated expression plasmids, *Ipl1/A1* and *Ipl1/A2*, containing the unique N-terminal domain of *Ipl1* (amino acids 1–101), fused to the C-terminal catalytic domain of *aurora1* (amino acids 75–344) or *aurora2* (amino acids 131–403), respectively. Additional *Ipl1/aurora* fusions were made in which the essential lysine at residue 106 (K106) of *aurora1* or residue 162 (K162) of *aurora2* was mutated to a methionine resulting in catalytically inactive forms of both proteins. These kinase-dead constructs were designated *Ipl1/A1KM* and *Ipl1/A2KM*, respectively. These coding regions were subcloned into a CEN vector (Sikorski and Hieter, 1989) under control of the native *Ipl1* promoter. The wild-type *Ipl1* construct complemented the *ipl1-1* mutation at 37°C, whereas no growth was observed with either the wild-type or kinase-dead fusions of *Ipl1/aurora1* or *Ipl1/aurora2* (Figure 2D). However, at the less restrictive temperature of 34°C, the *Ipl1/A2* fusion partially complemented the *ipl1-1* mutation, whereas the kinase-dead *Ipl1/A2KM* and all *aurora1* constructs failed to rescue the mutation (Figure 2C). Thus, in support of the conclusions derived from analysis of the primary amino acid sequence of these proteins, it appears that the *aurora2* kinase is structurally and functionally equivalent to *Ipl1*, whereas *aurora1* exhibits a biologically distinct activity.

Aurora1 and *aurora2* are cell cycle regulated

Based on the predicted involvement of *Drosophila* *aurora* and yeast *Ipl1* in centrosome separation and/or chromosome segregation, we investigated whether human *aurora1* and *aurora2* are cell cycle regulated. HeLa cells were synchronized at the G₁/S transition by a double thymidine/aphidicolin block (Golsteyn *et al.*, 1994) and followed through the completion of mitosis. After release from the G₁/S transition, the cells were analyzed for *aurora1* and *aurora2* RNA expression, protein expression and kinase activity. The DNA content at each time point was analyzed by flow cytometry (Figure 3A). *Aurora1* and *aurora2* RNA levels were low at the G₁/S transition (time = 0) and gradually increased as the cells progressed through S phase (time = 2–6 h) (Figure 3B, upper and middle panels) and through G₂ and mitosis (time = 8–10 h). *Aurora1* RNA levels were highest at 8–10 h after release, corresponding to the G₂ and M phases of the cell cycle (Figure 3B, upper panel). The amount of *aurora2* RNA peaked at 8 and 9 h post-release as the cells progressed from G₂ into mitosis and returned to low levels as the cells re-entered

G₁ at 12 h after release from the block (Figure 3B, middle panel). Actin RNA served as a loading control (Figure 3B, bottom panel). As expected, aurora1 and aurora2 proteins also varied during the cell cycle. Aurora1 and aurora2 proteins peaked at 8–11 h and 8–10 h after release, respectively (Figure 3C, upper and middle panels). p34^{cdc2} protein levels served as a loading control (Figure 3C, bottom panel). We also examined aurora1 and aurora2 kinase activity during cell cycle progression. The aurora1 kinase activity was maximal during mitosis at 10–11 h after release (Figure 3D, top panel). Aurora2 kinase activity peaked at 9 h after release (Figure 3D, middle panel). p34^{cdc2} kinase activity, which served as a marker for mitosis, peaked at 10 h after release (Figure 3D, bottom panel). Thus, both aurora1 and aurora2 RNA, protein and kinase activity were cell cycle-regulated, all being maximal during G₂ and mitosis. Aurora2 kinase activity was highest just prior to maximal activation of aurora1 and p34^{cdc2}. These data suggest that aurora2 function precedes that of aurora1 in mitosis.

Aurora1 and aurora2 are localized to mitotic structures

The subcellular location of endogenous aurora1 and aurora2 was determined by indirect immunofluorescence. Exponentially growing HeLa cells were fixed with methanol and probed with a monoclonal antibody to α -tubulin and with protein A affinity-purified antibodies to either aurora1 (Figure 3E, bottom panel) or aurora2 (Figure 3F, bottom panel). The aurora1 and aurora2 antibodies only stained structures in mitotic cells and did not stain any recognizable structures or compartments in interphase cells. This is understandable given that the proteins are most abundant at this stage of the cell cycle (Figure 3C). In anaphase and early telophase, the aurora1 antibodies stained the midzone and telophase disc (Andreassen *et al.*, 1991), whereas in late telophase and early G₁, they stained the post-mitotic bridge (Figure 3E, bottom panel). In metaphase and anaphase, the aurora2 antibodies stained the centrosome, spindle poles and the spindle (Figure 3F, bottom panel), whereas in telophase cells the aurora2 antibodies primarily stained the spindle pole (Figure 3F, bottom panel). The aurora2 immunostaining is consistent with that reported elsewhere (Gopalan *et al.*, 1997; Kimura *et al.*, 1997). The subcellular localization of aurora1 and aurora2 suggests that aurora1 may function later in mitosis than aurora2. This supports the observation that, in synchronized cells, aurora2 kinase activity precedes that of aurora1 (Figure 3D). In addition, the localization of aurora1 and aurora2 to mitotic structures confirms that they are indeed likely to be involved in chromosome segregation.

Expression of aurora1 and aurora2 RNA

Northern blot analysis of mRNA isolated from normal adult human tissues demonstrates that *aurora2* expression is primarily restricted to testis, thymus and fetal liver (Figure 4A), with very weak expression in bone marrow, lymph node and spleen, and no detectable expression in all other adult tissues examined. Human *aurora1* was also expressed at highest levels in normal thymus and fetal liver, with a moderate level of expression in lung and small intestine (Figure 4A).

Fig. 4. Expression of human *aurora1* and *aurora2*. (A) Northern blot containing poly(A)⁺ mRNA (2 µg per lane) from normal human tissue hybridized with an *aurora1* or *aurora2* DNA probe. (B) *Aurora2* Northern blots containing total RNA (20 µg) from human tumor cell lines. The single 2.4 kb *aurora2* transcript is marked. RNA from the lung cancer cell line NCI-H23 was included as a standard for the tumor blots. Cell lines included are: 1, HT-29; 2, HCC-2998; 3, COLO 205; 4, HCT-15; 5, KM012; 6, UO-31; 7, SN12C; 8, CAKI-1; 9, RXF393; 10, ACHN; 11, 786-0; 12, TK-10; 13, LOX IMVI; 14, SK-MEL-2; 15, SK-MEL-5; 16, SK-MEL-28; 17, UACC-62; 18, UACC-257; 19, M14; 20, MCF-7/ADR-RES; 21, HS 578T; 22, MDA-MB-435; 23, MDA-N; 24, T-47D. (C) *Aurora2* Northern blot containing total RNA (10 µg) from cultured primary human endothelial and epithelial cells. The single 2.4 kb *aurora2* transcript is marked. RNA from the lung cancer cell line NCI-H23 was included as a standard for the blots. Primary cell RNAs are: 1, coronary artery endothelial cells; 2, pulmonary artery endothelial cells; 3, lung microvascular endothelial cells; 4, dermal microvascular endothelial cells; 5, mammary epithelial cells; 6, renal proximal tubule epithelial cells; and 7, renal cortex epithelial cells.

Since *aurora2* was highly represented in the initial PCR screen of primary colon tumors, we examined the expression of *aurora2* RNA in a panel of 25 human tumor cell lines of lung, colon, renal, melanoma and breast origin. The 2.4 kb *aurora2* transcript was expressed at high levels in 96% (24 of 25) of these transformed cell lines (Figure 4B), with the only exception being the UO-31 renal carcinoma cell line. Our preliminary analysis revealed that the 1.4 kb *aurora1* transcript was also expressed in the same 24 tumor cell lines (unpublished data), although we have yet to examine this in more detail. We also saw modest, but detectable, expression of *aurora2* in a panel of RNAs isolated from cultured primary epithelial and endothelial cells (Figure 4C). We conclude that *aurora2* is preferentially expressed in all rapidly

dividing cells, but its levels are significantly up-regulated in a broad range of tumor cell lines.

Amplification and overexpression of *aurora2* in primary human colorectal cancers

The *aurora2* gene was mapped using the Stanford Human Genome Center G3 radiation hybrid panel. Human *aurora2* is located on chromosome 20q13.2 (LOD score of 17.26 to linked marker SHGC-3245). Mapping was also confirmed by hybridization to a human-rodent somatic cell hybrid panel (Coriell Cell Repository, Camden, NJ). *Aurora2* maps adjacent to the vitamin D hydroxylase (*CYP24*) gene and the cosmid probe RMC20C001 that lie at 0.825–0.83 Flpter (fractional length from pter) on chromosome 20 (Tanner *et al.*, 1994, 1996). Both of these markers have been characterized for their presence in the 20q13 amplicon common to many human malignancies, particularly those from breast, bladder and colon cancers (Muleris *et al.*, 1987; Bigner *et al.*, 1988; Yaseen *et al.*, 1990; Kallioniemi *et al.*, 1994; Tanner *et al.*, 1994, 1996; Iwabuchi *et al.*, 1995; Schlegel *et al.*, 1995; Bockmuhl *et al.*, 1996; Courjal *et al.*, 1996; Reznikoff *et al.*, 1996; Solinas-Toldo *et al.*, 1996; James *et al.*, 1997; Larramendy *et al.*, 1997).

Since the *aurora2* gene maps to a prevalent tumor amplicon, we questioned whether the *aurora2* gene was also amplified in a cohort of primary human colorectal tumors and matched normal colorectal tissue from the same patients. Southern blot hybridization was performed using an *aurora2* cDNA probe along with a control probe for the *CYP24* gene that serves as a marker of the amplicon (Tanner *et al.*, 1994, 1996). The *aurora2* probe hybridized to *Pst*I fragments of 5.8, 3.7, 3.3, 2.8, 2.5 and 1.3 kb. The 5.8, 3.3, 2.8 and 2.5 kb bands are specific to *aurora2*, while the 3.7 and 1.3 kb bands represent cross-hybridization to the *aurora3* and *aurora4* pseudogenes which map to chromosomes 1 and 10, respectively. Only the *aurora2*-specific bands showed amplification in the tumor samples. *Aurora2* DNA was amplified in (52%) 41 of 79 of the primary colorectal tumors for which suitable DNA was available for genotyping (Figure 5B). The *CYP24* gene was found to be co-amplified with *aurora2* in (90%) 37 of 41 matched pairs and was found only once to be amplified in the absence of *aurora2* amplification.

Aurora2 RNA levels were characterized by Northern blot analysis of samples from the same panel of matched tumor/normal tissues. Approximately 54% (22 of 41) of the tumors showed increased expression of the 2.4 kb *aurora2* transcript as compared with the normal colon control. *Aurora2* RNA showed 4- to 28-fold overexpression in tumor versus normal tissue. Representative Northern and Southern data from 12 matched tumor/normal pairs are shown in Figure 5, where nine samples demonstrated amplification of *aurora2* DNA in the range of 2- to 8-fold in the tumors compared with normal tissue (2164, 2172, 2193, 3204, 2255, 3189, 3191, 3193 and 2176) and three samples (1985, 2175 and 2257) showed no amplification. This level of *aurora2* amplification is consistent with other reports of 1.5- to 10-fold increases in copy number of 20q13 in primary tumors and tumor cell lines (Kallioniemi *et al.*, 1994; Tanner *et al.*, 1996; Sen *et al.*, 1997). Sample 3193 still shows a relative level of DNA amplification after adjusting for unequal sample loading. One of the

samples (1985) clearly demonstrates RNA overexpression in the absence of DNA amplification, whereas the other 11 show a direct correlation between DNA amplification and RNA overexpression. We obtained complete data for analysis from 37 matched sets of RNA and DNA from both normal and tumor samples. Data in Table I show a high correlation ($\rho = 0.695$, Pearson correlation; $P < 0.00003$, Fisher's exact test) between *aurora2* DNA amplification and RNA overexpression with only one discordant result. In the single case of *aurora2* DNA amplification in the absence of RNA overexpression, *aurora2* RNA was actually elevated in both the normal and tumor specimens, compared with other tumor/normal pairs. It is conceivable that high expression of *aurora2* RNA in this normal colon sample may represent an early predisposing lesion. Conversely, five paired samples showed increased RNA expression in the absence of DNA amplification, possibly due to transcriptional activation. If these five pairs are excluded from the analysis, the correlation between *aurora2* DNA amplification and RNA overexpression increases to $\rho = 0.939$. These data suggest that DNA amplification is a mechanism for *aurora2* activation and also implicates *aurora2* as an oncogene at 20q13 whose high level amplification correlates with poor clinical outcome in breast cancer (Isola *et al.*, 1995).

To determine if the *aurora2* sequence from the 20q13 amplicon was the same as that from normal sources, we performed direct sequencing of RT-PCR products encompassing the complete *aurora2* coding region from 10 primary colorectal tumor samples. Eight samples, including tissues with both normal and amplified levels of the 20q13 amplicon, confirmed the *aurora2* sequence. A single base change was identified in two samples (1985 and 2193) resulting in a phenylalanine to isoleucine change at residue 31 in the N-terminal Aurora Box1 (circled in Figure 1A). Experiments are planned to determine if this is simply a polymorphism or whether this change affects *aurora2* activity. Nonetheless, these analyses demonstrate that the 20q13 amplicon typically contains increased copies of the intact, unmutated *aurora2* coding region.

Detection of *aurora2* protein in primary human colon cancer samples

To determine whether the amplification of *aurora2* gene and message resulted in overexpression of the protein, anti-*aurora2* antibodies were used to probe blots of protein lysates made from cryostat sections of primary human colon carcinomas or from adjacent normal tissue isolated from the same patient. As shown in Figure 6A, the *aurora2* antibodies detected a protein of ~46 kDa in the two primary human colon carcinomas, but not in samples derived from the adjacent normal tissue. Due to the limited amount of tissue available, we were unable to determine if *aurora2* was amplified in these samples. These antibodies also detected overexpression of *aurora2* protein in various cultured tumor cell lines derived primarily from colorectal carcinomas (Figure 6B). While most tumor cell lines examined expressed detectable levels of *aurora2* protein, others including A549 cells do not (Figure 6B, lane 1).

***Aurora2* transforms Rat1 fibroblasts**

If *aurora2* is a relevant target on the 20q13 amplicon, one might expect that overexpression of *aurora2* would be

Fig. 5. *Aurora2* RNA overexpression and DNA amplification in primary human colorectal cancers. (A) Northern blots containing total RNA (6 μ g per lane) from primary human colorectal cancers and from matched normal colon controls were hybridized with an *aurora2* DNA probe. Blots were stripped and reprobed with a human β -actin probe to confirm equivalency and quality of RNA loading. (B) Southern blots containing 5 μ g of *Pst*I-digested DNA per lane from primary human colorectal cancers and from matched normal colon controls were hybridized with an *aurora2* DNA probe (pSG19). The location of the 2.8 and 2.5 kb *aurora2* fragments are marked. Blots were stripped and reprobed with a human β -globin probe to confirm equal loading. Patient numbers are shown for each of the matched sets of normal (N) and tumor (T) tissue.

Table I. Amplification of *aurora2* DNA correlates with RNA overexpression

Sample	Amount DNA amplified or RNA overexpressed
DNA	52% (41/79)
RNA	54% (22/41)
DNA and RNA	43% (16/37)
DNA w/o RNA	3% (1/37)
RNA w/o DNA	14% (5/37)

Matched tumor/normal pairs of colorectal tissue were analyzed by Southern blot for amplification of *aurora2* DNA and by Northern blot for overexpression of *aurora2* RNA. Data are presented for the 79 DNA samples and 41 RNA samples analyzed. Both RNA and DNA samples were available for 37 matched tumor/normal pairs. DNA w/o RNA, refers to DNA amplification without RNA overexpression.

transforming. To examine this question, we established stable NIH 3T3 and Rat1 cell lines that express human *aurora2*. Rat1 cells were infected with retroviruses that express a hemagglutinin (HA)-tagged (Pati, 1992) wild-type *aurora2* or a kinase-inactive mutant where the essential lysine at residue 162 (Figure 1A) was changed to a methionine (K162M). In addition, an activating mutation was made in which the threonine at residue 288 (Figure 1A) in the activation loop was changed to an aspartic acid (T288D). This mutation was designed to mimic constitutive phosphorylation at this site and the recombinant T233D *aurora2* protein demonstrated increased specific activity when expressed in bacteria or baculovirus-infected insect cells (unpublished data). Several clones expressing each construct were selected that expressed similar amounts of *aurora2* protein (Figure 7A, lanes 2–4).

To characterize the transforming potential of *aurora2*, we performed soft agar assays with the Rat1 clones. The vector control, K162M, wild-type and T288D *aurora2*-expressing Rat1 cells were plated in soft agar and scored for growth after 4 weeks. As shown in Figure 7B, cells expressing the wild-type and the T288D *aurora2* formed colonies in soft agar, in contrast to the lack of growth by cells expressing the kinase-inactive *aurora2*. Ten of 13

Fig. 6. *Aurora2* protein levels are elevated in tissue and cell lines derived from colon carcinomas. (A) Total protein lysates were prepared from matched samples of tumor and adjacent normal tissue. Equal amounts of protein from each pair was loaded on a 12% gel and immunoblotted with affinity-purified antibodies to *aurora2*. Lane 1, 72 μ g of total protein isolated from normal colon epithelium adjacent to tumor HT374; lane 2, 72 μ g of total protein isolated from tumor HT374; lane 3, 90 μ g of total protein isolated from normal tissue adjacent to tumor HT376; lane 4, 90 μ g of total protein isolated from tumor HT376. N, normal tissue; T, tumor tissue. (B) Total protein lysates were prepared from six colon adenocarcinoma cell lines (LS180, HCT-15, HT-29, COLO 205, SW480 and SW948), one rectal adenocarcinoma cell line (SW837), one primary cecum carcinoma cell line (SNU-C2B) and one lung carcinoma cell line (A549). 50 μ g of total protein were loaded in each lane on a 12% gel and immunoblotted with affinity-purified antibodies to *aurora2*.

wild-type clones and six of 12 T288D clones grew in soft agar, compared with one of 11 vector and K162M clones. We quantitated the number of colonies formed in soft agar

Fig. 7. Aurora2 transforms Rat1 fibroblasts. **(A)** Rat1 cells were transfected with either pLXSN (vector), HA-tagged kinase-inactive (K162M) *aurora2*, HA-tagged wild-type *aurora2*, or HA-tagged activated (T288D) *aurora2*. Cell lysates from stable Rat1 clones were immunoprecipitated with a monoclonal antibody specific to the HA tag. Immune complexes were resolved directly on a 12% SDS–polyacrylamide gel, transferred to a nylon membrane and probed with protein A-purified antibodies to aurora2. Lane 1, vector control; lane 2, kinase-inactive *aurora2* (K162M); lane 3, wild-type *aurora2*; lane 4, activated *aurora2* (T288D). **(B)** Stable Rat1 cell lines expressing either kinase-inactive (K162M), wild-type or activated (T288D) *aurora2* were plated on soft agar and incubated at 37°C for 4 weeks. ‘Vector’ represents Rat1 cells stably infected with pLXSN. **(C)** NIH 3T3 cells expressing the kinase-inactive (K162M) or activated *aurora2* (T288D) constructs were injected subcutaneously between the scapula of *nu/nu* mice. Tumor size was measured at 4, 6 and 8 weeks post-implantation.

from two independent clones of each of the transfections. The average number of colonies per 200 000 cells plated were: K162M, 32 colonies; wild-type, 470 colonies; and T288D, 250 colonies. Although the T288D Rat1 stables formed fewer colonies than the wild-type *aurora2* Rat1 stables, the T288D colonies in general grew to larger size (Figure 7B). None of the Rat1 stables showed altered growth characteristics as compared with the vector control; however, the NIH 3T3 T288D clones did grow to a higher density than the vector control, wild-type and K166M clones. The T288D *aurora2* mutant was also able to transform NIH 3T3 cells, as measured by growth in soft agar (unpublished data) and growth as tumors in nude mice (Figure 7C). Apparently, *aurora2* kinase activity was required for cellular transformation, as the kinase-inactive *aurora2* (K162M) failed to stimulate the growth of Rat1 or NIH 3T3 cells in soft agar or as tumors in nude mice.

Discussion

In this report we characterize a new family of two human serine/threonine kinases designated aurora1 and aurora2. Using drug release protocols to synchronize HeLa cells, we found that *aurora1* and *aurora2* RNA levels, protein levels and kinase activities were low during interphase, but increased as cells proceed into mitosis. These activity profiles are similar to that of cell cycle-regulated p34^{cdc2}, however, *aurora2* activity peaks 1 h before p34^{cdc2}, and the *aurora1* kinase activity persists for 1 h after p34^{cdc2}. The activity of p34^{cdc2} has been shown to maximal in

metaphase, suggesting that *aurora2* kinase activity peaks in prometaphase while *aurora1* kinase activity is high from metaphase through telophase. Increased *aurora2* protein levels cannot completely account for its increased kinase activity. Immunoblot analysis demonstrates equivalent amounts of *aurora2* protein in samples prepared 9 and 10 h after release from a double thymidine/aphidicolin block (Figure 3C, middle panel), but the amount of *aurora2* kinase activity is three times greater at the earlier time point (Figure 3D, middle panel). This raises the question of whether *aurora2* kinase activity is also regulated by a post-translational mechanism, possibly by phosphorylation of Thr288 in the activation loop.

The *aurora1* protein was localized to the midzone of cells in anaphase and to the post-mitotic bridge and midbody during telophase, while *aurora2* protein was localized to the mitotic spindle of cells in metaphase and anaphase. These findings support the biochemical studies which suggest that both are cell-cycle-regulated proteins and that *aurora2* functions earlier in mitosis than *aurora1*. A recent manuscript on *AIM-1*, the rat orthologue of *aurora1*, also describes its localization to the midbody region during telophase and presents evidence to suggest that *aurora1* is required for cytokinesis, since its disruption results in polyploidy and decreased viability (Terada *et al.*, 1998).

We have shown that *aurora2* RNA is expressed in a variety of human tumor cell lines while having limited expression in normal human tissue. The *aurora2* gene was mapped to chromosome 20q13, a region frequently

amplified in human tumors. *Aurora2* DNA was found to be amplified, and its RNA overexpressed, in 52% of a cohort of primary colorectal cancers examined. In addition, we demonstrate that overexpression of the *aurora2* kinase transforms rodent fibroblasts.

Gene amplification in tumor cells is often characterized by the presence of cytogenetic aberrations including heterogeneous staining regions (HSRs) and double minute chromosomes. Recently, the technique of comparative genomic hybridization (CGH) has provided a sensitive means by which to identify tumor-associated amplicons (Kallioniemi *et al.*, 1992; Tanner *et al.*, 1994, 1996). While several oncogenes have been shown to be amplified in human tumors (Slamon *et al.*, 1987; van de Vijver, 1993), including *HER2* (17q12), *myc* (8q24) and *cyclin D* (11q13), most of these genes are not contained within some of the more prevalent amplicons (Tanner *et al.*, 1994, 1996).

The most common regions of high-copy amplification in human breast cancer have been localized to 17q22 and 20q13.2 (Kallioniemi *et al.*, 1994; Tanner *et al.*, 1994, 1996). Low-level amplification of 20q has been described in 6–18% of primary breast cancers and 40% of breast cancer cell lines and the incidence increases to 60% in BRCA2-positive breast cancers (Kallioniemi *et al.*, 1994; Tanner *et al.*, 1994, 1996; Tirkkonen *et al.*, 1997). High levels of 20q amplification also correlate with poor prognosis for patients with node-negative breast cancer (Isola *et al.*, 1995). Low-level amplification of 20q has also been noted in colon cancer, ovarian cancer, bladder cancer, gliomas, medulloblastomas, chondrosarcomas, pancreatic tumors and head and neck cancers (Muleris *et al.*, 1987; Bigner *et al.*, 1988; Yaseen *et al.*, 1990; Iwabuchi *et al.*, 1995; Schlegel *et al.*, 1995; Bockmuhl *et al.*, 1996; Courjal *et al.*, 1996; Reznikoff *et al.*, 1996; Solinas-Toldo *et al.*, 1996; James *et al.*, 1997; Larramendy *et al.*, 1997). Relevant to this manuscript, several studies have found chromosomal gains of 20q in ~60% of primary colorectal carcinomas (Muleris *et al.*, 1987; Yaseen *et al.*, 1990; Schlegel *et al.*, 1995). Cell culture models have suggested that low-level amplification of 20q is associated with immortalization and subsequent high-level amplification correlates with chromosomal instability (Savelieva *et al.*, 1997).

Recently, the *CAS* (cellular apoptosis susceptibility) gene has been localized to the 20q13.2 amplicon (Brinkmann *et al.*, 1996). *CAS* is a human homologue of the yeast *CSE1* gene and functions as a transport factor to bind importin- α and mediate its transport from the nucleus back into the cytoplasm (Kutay *et al.*, 1997). While *CAS* is amplified or translocated in a variety of tumor cell lines, it is amplified in only a restricted number of primary tumors and does not appear to be the primary target of the amplicon (Tanner *et al.*, 1994, 1996).

Aurora2 maps adjacent to the *CYP24* gene and the cosmid probe RMC20C001 that have been proposed to reside within the center of the 20q13.2 amplicon (Tanner *et al.*, 1994, 1996). Southern analysis confirms the tight linkage of *aurora2* to the amplicon. However, unlike *CYP24* and *CAS*, the amplification of *aurora2* DNA also correlates with overexpression of its transcript. A recent report describes a search for expressed transcripts encoded by the 20q amplicon in a panel of three breast cancer cell

lines, leading to the identification of a fragment of the *aurora2* gene (Sen *et al.*, 1997). This report further confirms our localization of *aurora2* to this critical region. Furthermore, we demonstrate the transforming effects of the *aurora2* kinase, making it a strong candidate for an oncogene on the 20q13 amplicon which is predicted to play a role in a wide variety of epithelial tumors. We show here that the wild-type *aurora2* transforms Rat1 fibroblasts. However, this same construct has no effect on NIH 3T3 transformation, whereas the activated *aurora2* mutant was able to transform both cell lines. A similar selectivity in cellular transformation has been reported for the serine/threonine kinase Pak1, which plays a role in ras-dependent transformation of Rat1 fibroblasts, but not of NIH 3T3 cells (Tang *et al.*, 1997). It is conceivable that the presence of normal cell cycle checkpoints in NIH 3T3 cells prevents the activation of the *aurora2* protein. Alternatively, it is possible that overexpression of *aurora2* is transforming only in the presence of co-amplification or mutation in additional genes.

Identification of *aurora2* as a transforming protein expands a rather limited list of serine/threonine kinase (STK) oncogenes. While numerous cellular proto-oncogenes have been found to encode tyrosine kinases, only a few are found to be STKs including: raf, mos, pim1, cot, mek, akt and PLK1 kinases (Van Beveren *et al.*, 1981; Selten *et al.*, 1986; Beck *et al.*, 1987; Miyoshi *et al.*, 1991; Bellacosa *et al.*, 1993; Cowley *et al.*, 1994; Smith *et al.*, 1997). Many of these proteins are involved in signaling through MAP kinase pathways (raf, mos, cot and mek), whereas pim1 cooperates with c-Myc and akt may be involved in the oncogenic signal transduced by PI3 kinase (Hunter, 1997; Kinzler and Vogelstein, 1997). Both PLK1 and *aurora2* are cell-cycle-regulated STKs and provide the first example to suggest a link between centrosome integrity and cellular transformation.

Protein phosphorylation plays a central role in regulating cell cycle progression and in the process of centrosome separation and chromosome segregation (Hoyt *et al.*, 1991; Elledge, 1996; Sherr, 1996; Weiss and Winey, 1996; Taylor and McKeon, 1997). Until recently, no compelling connection has been made between the proteins involved in this process and cancer. The observation that mRNA levels of human *PLK1* are elevated in a majority of non-small cell lung carcinomas and that high levels correlate with poor prognosis (Wolf *et al.*, 1997), implies either a causal or symptomatic role in cancer for proteins involved in the centrosome regulation. Microinjection of anti-PLK1 antibodies into HeLa cells and normal diploid Hs68 fibroblasts results in an inhibition of centrosome maturation and a block in mitosis (Lane and Nigg, 1996, 1997). In addition, the disruption of PLK1 by these antibodies leads to the formation of abnormal nuclei in HeLa cells, but not in Hs68 cells, suggesting that a checkpoint which monitors centrosome maturation in normal cells may be absent in tumor cells (Lane and Nigg, 1996, 1997). Furthermore, constitutive overexpression of PLK1 transforms NIH 3T3 cells (Smith *et al.*, 1997). The genetics of *Drosophila* *aurora* and the yeast Ipl1 protein kinases suggest they may be involved in PLK1/polo/Cdc5p pathway, or in a related parallel cascade. Indeed, the cell cycle regulation and the intracellular localization of the human *aurora1* and *aurora2* kinases are similar to those of

PLK1. It is conceivable that these three proteins form a centrosome-associated kinase cascade whose disruption leads to genomic instability and chromosome segregation defects. It will be important to determine whether *aurora2* amplification results in a compensatory increase in PLK1 or *aurora1* expression and if any of these proteins serve as substrates for the others.

Materials and methods

Molecular cloning

Degenerate oligonucleotide primers were designed for PCR cloning based on kinase domains I and IX of CCK4 (DDBJ/EMBL/GenBank accession No. U33635) (Mossie *et al.*, 1995), a receptor tyrosine kinase expressed in a wide range of normal and transformed epithelial cells. The sense primer was 5'-GARTTYGGNGARGTNTTYTNGC-3', encoding the amino acids EFGEVFLA and the antisense primer was 5'-AGNACN-CCRAANGCCACACRTC-3', encoding the complementary strand of amino acids DVWAFGLV. These primers were applied to ssDNA generated from RNA isolated from several colon cancer cell lines as well as other tumor sources. PCR products of 500–600 bp were subcloned and sequenced, revealing a fragment related to *Drosophila aurora*. This fragment was used to probe a lambda library constructed from a pool of several human pancreatic cancer cell line RNAs, leading to isolation of full-length clones for human *aurora1*. Two weakly hybridizing clones were also isolated and sequence analysis revealed that they represented a related but distinct cDNA termed *aurora2*. Full-length clones were also isolated for both genes from normal human duodenum cDNA. All clones were sequenced on both strands with internal oligonucleotide primers using both T7 polymerase manual sequencing and using dye-terminator cycle sequencing with AmpliTaq DNA polymerase on an ABI Prism 377. The complete *aurora2* coding sequence was also confirmed from 10 primary colorectal tumor samples. Primers 5'-CGCCTTTCATCCGCTCCTG-3' and 5'-GATTGCTCCTGTG-AAGAC-3' were used in an RT-PCR with ssDNA generated from the tumor RNAs. The PCR products were purified by GeneClean and sequenced directly by dye-terminator cycle sequencing with several oligonucleotide primers. While no sequence differences were observed between clones isolated from normal or tumor sources, we did identify a single nucleotide polymorphism in two of the tumor samples which would encode an F to I change at residue 31. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. Abbreviations for degenerate nucleotide residues are: R = A or G; Y = C or T; N = A, C, G or T.

Generation of *Ipl1/aurora* fusion constructs

DNA encoding the N-terminal 101 amino acids of *S.cerevisiae* Ipl1 was fused to a fragment encoding the C-terminal 270 amino acids (75–344) of human *aurora1*, or to the C-terminal 273 amino acids (131–403) of human *aurora2*, to generate plasmids Ipl1/A1 (pSG125) and Ipl1/A2 (pSG121), respectively. Kinase-dead forms of both plasmids were constructed by oligonucleotide-directed mutagenesis to convert a Lys to Met at the ATP binding site in the catalytic domains, generating Ipl1/A1KM (pSG129) and Ipl1/A2KM (pSG1260). A full-length Ipl1 construct (pSG128) and the *IPL/aurora* fusions were subcloned into a low-copy CEN URA3 plasmid (Sikorski and Hieter, 1989) under the control of the *Ipl1* native promoter. A hemagglutinin epitope (Pati, 1992) was fused to the C-terminus of all the constructs. Cell lysates were prepared from the wild-type yeast strain, CY184 (Zhu *et al.*, 1995) transformed with the individual expression plasmids and recombinant proteins were detected by Western analysis with an anti-HA antibody.

Complementation in yeast

The Ipl1 and the AUR expression plasmids were transferred into the *ipl1-1 ts* mutant strain, CCY464-1D (Francisco *et al.*, 1994) and selected on synthetic medium minus uracil (SC-URA) (Sherman *et al.*, 1974). Single transformants were isolated and restreaked in triplicate onto the same medium and incubated for three days at 26°C, 34°C or 37°C.

Antibodies

Rabbits were injected with either a peptide consisting of the 12 C-terminal amino acids of *aurora1* conjugated to KLH or a purified GST fusion

protein containing the entire coding sequence of *aurora2*. Bleeds were tested for their ability to immunoprecipitate *in vitro*-translated ³⁵S-labeled *aurora1* and *aurora2*, respectively. *Aurora1*- or *aurora2*-specific immune sera were partially purified by protein A affinity chromatography and frozen in small aliquots. The *aurora1* antisera immunoprecipitated a ~40 kDa protein from HeLa cells, close to the predicted molecular weight of 39.4 kDa, and was competed by the *aurora1* peptide (unpublished data). The *aurora1* antisera also recognized a 40 kDa protein by immunoblotting of total HeLa cell lysates (unpublished data). The *aurora2* antisera immunoprecipitated a ~48 kDa protein from HeLa cells, close to the predicted molecular weight of 45.8 kDa, that was not detected by the preimmune serum. The *aurora2* antisera also recognized a 48 kDa protein by immunoblotting of total HeLa cell lysates. *In vitro* kinase assays demonstrate that the *aurora1* and *aurora2* immune complexes both contained a kinase capable of phosphorylating MBP, α -casein and protein kinase A (GST2TK, a GST vector containing a PKA phosphorylation site), but not histone H1 (unpublished data).

Cell cycle analysis

Ten cm tissue culture dishes were seeded at a density of 3×10^6 with exponentially growing HeLa cells. The following day, thymidine (Sigma) was added to the media to a final concentration of 2 mM and the plates were incubated for 14 h at 37°C. The plates were then washed three times with phosphate-buffered saline (PBS) and normal growth media was added. Following 11 h at 37°C, aphidicolin (Sigma) was added to a final concentration of 1 μ g/ml and the plates were incubated at 37°C for an additional 14 h. Plates were then washed three times with PBS followed by the addition of normal growth media. This time point was designated time zero. Flow cytometry was as previously described (Bischoff *et al.*, 1990). Total RNA was resolved on a 1.2% agarose gel and transferred to a nylon membrane (Amersham). Full-length *aurora1* and *aurora2* cDNAs were ³²P-labeled by random priming (Stratagene) and used to probe Northern blots. Total protein for immunoblots and kinase assays were isolated as follows: HeLa cells were solubilized in kinase lysis buffer (50 mM HEPES pH 7.4, 100 mM KCl, 25 mM NaF, 0.5% NP-40, 1 mM Na₃VO₄, 1 mM DTT and protease inhibitors) for 15 min on ice, spun in a microfuge at 10 000 g for 10 min at 4°C. The resulting supernatant was transferred to a clean tube and the total protein concentration was determined by Bradford analysis. Equal amounts of protein, 50 μ g and 500 μ g, were loaded on gels for immunoblots or immunoprecipitated for kinase assay, respectively. The immune complexes were washed three times with kinase lysis buffer followed by three washes with kinase buffer (without [γ -³²P]ATP and artificial substrate) and resuspended in 30 μ l of 1 $\times$ kinase buffer [20 mM HEPES pH 7.4, 150 mM KCl, 5 mM MnCl₂, 5 mM NaF, 1 mM DTT, 50 μ M ATP, 20 μ M [γ -³²P]ATP and 0.5 mg/ml myelin basic protein, GST2TK protein (Pharmacia) or histone H1 (Boehringer Mannheim)]. *In vitro* kinase reactions were carried out for 20 min at 37°C and stopped by the addition of 30 μ l of 2 $\times$ Laemmli SDS sample buffer. Samples were incubated for 5 min at 95°C and resolved on 14% SDS-polyacrylamide gels.

Immunofluorescence

HeLa cells were plated on coverslips at ~25% confluency. The following day the cells were washed once with ice-cold PBS and fixed with methanol at -20°C overnight. The cells were washed three times with ice-cold PBS followed by a 5 min incubation at room temperature in PBS containing 0.05% Triton X-100. The permeabilized cells were washed three times with ice-cold PBS and then covered with a solution of 10% non-fat milk in PBS and incubated for 30 min at 37°C in a humidified chamber. The antibodies, α -*aurora1* and α -*aurora2* and α -tubulin, were diluted in PBS containing 10% non-fat milk, placed as a drop on the coverslips, and incubated for 30 min at 37°C in a humidified chamber. The coverslips were then washed six times with PBS and covered with a solution containing goat anti-rabbit-FITC (Santa Cruz Biotech), goat anti-mouse Rhodamine (Sigma) and 1 μ g/ml DAPI (Boehringer Mannheim) for 30 min in the dark at 37°C in a humidified chamber. The coverslips were then washed six times with PBS and attached to slides with clear nail polish.

Northern blots

Cell pellets from cultured tumor cell lines were provided by Nick Scudero (Developmental Therapeutics Program, NCI) and are part of the NCI tumor panel (see website listing at http://epnws1.ncicrf.gov:2345/dis3d/cancer_screen/celllist.html). Primary human endothelial and epithelial cells were obtained from Clontech. Normal human tissue samples were obtained from the Cooperative Human Tissue Network (Cleveland,

OH). Human colorectal tissue samples for Northern and Southern analysis were obtained from Los Angeles area hospitals including UCLA-Harbor, Wadsworth and Cedars Sinai from 1988 to 1997. Tumor histology was confirmed prior to preparing RNA, DNA and protein lysates from each sample. Total cell or tissue RNA was isolated using the guanidine salts/phenol extraction protocol of Chomczynski and Sacchi (1987). Northern blotting was performed using standard techniques (Peles *et al.*, 1997) with a random-labeled 586 bp *Bam*HI–*Ssp*I fragment of the human *aurora2* cDNA. A multiple tissue Northern blot and a human immune system blot (Clontech) containing 2 µg poly(A)⁺ mRNA per lane were also probed for *aurora2* expression. A human β-actin cDNA probe (Clontech) was used to confirm equivalent loading of intact RNA. RNA (10 µg) from the NCI-H23 lung cancer cell line served as an internal standard for detection of *aurora2* expression on each blot. Blots were quantitated using a phosphorimager and ImageQuant software (Molecular Dynamics, Mountain View, CA).

Chromosomal localization

The Stanford G3 radiation hybrid panel was obtained from Research Genetics (Huntsville, Alabama). *Aurora2* primers used for radiation hybrid mapping were: 5'-CAGGGCTGCCATATAACCTGA-3' and 5'-CTAGCACAGGCTGACGGGGC-3'. The *aurora2* primers amplify a 255 bp fragment from the 3' UTR following a 25-cycle PCR with a 54°C annealing temperature. The raw score for *aurora2* against the SHGCR G3 panel is: 100000000010001010000001000100100000000010000001100001001010000010010010010010.

Southern blotting

Genomic DNA was isolated from the human colorectal tissue samples by standard methods (Proteinase K digestion, phenol:chloroform extraction and ethanol precipitation). Southern blots were prepared by digesting 5 µg of DNA with *Pst*I, separating the fragments on 1% agarose gels, blotting onto nylon membranes (Nytran-Plus, Schleicher & Schuell) and probing sequentially with a random primer-labeled 1044 bp *aurora2* cDNA fragment (pSG19) and a 1700 bp cloned fragment of the *CYP24* gene (pKS-h24; from J.Omdahl, University of New Mexico). A probe for human β-globin was used to confirm equivalent sample loading. Final washes were at 0.1× SSC, 0.1% SDS, 60°C. Autoradiographs were quantitated relative to β-globin using ImageQuant software (Molecular Dynamics, Mountain View, CA).

Statistical analysis

Statistical significance of the correlation between DNA amplification and RNA overexpression was calculated using Pearson correlation and the one-tailed Fisher's exact test using SAS Release 6.12.

Western blotting

Matched human tissue samples from primary colorectal carcinomas and adjacent normal tissue were obtained from the Cooperative Human Tissue Network (Cleveland, OH) and Pathology Associates International (Frederick, MD). Thirty µm cryostat sections of OCT-embedded tissue was lysed directly in 25 µl of ice-cold RIPA buffer (50 mM Tris–Cl pH 8.0, 150 mM NaCl, 1.0% NP-40, 0.5% deoxycholate, 0.1% SDS, 1 mM DTT and protease inhibitors) by gentle mixing on ice for 20 min. The lysate was then spun for 10 min at 10 000 g in a microfuge at 4°C. The resulting supernatant was transferred to a clean tube and the total protein concentration determined by Bradford analysis. Equal amounts of total protein from the matched samples were resolved on a 12% polyacrylamide gel, transferred to a nylon membrane (BioRad) and probed with a 1:2000 dilution of protein A-purified antibodies to *aurora2*. The immunoblot was developed with ECL reagent (Amersham). Lysates from tumor cell lines were prepared and analyzed as described above.

Expression constructs

HA-tagged (Pati, 1992) versions of wild-type, kinase-dead (K162M) and activated (T288D) *aurora2* were subcloned into the expression vector pLXSN. These constructs were transfected into the amphotropic packaging cell line PA317 and the supernatants were harvested and used to infect the producer cell line GP+E-86 (Markowitz *et al.*, 1988). Neomycin-resistant clones were selected and assayed for *aurora2* protein expression (unpublished data). Supernatants from the positive producer cell lines were used to infect Rat1 and NIH 3T3 cells. Stable clones were selected for in the presence of neomycin and assayed for *aurora2* protein expression by immunoblotting.

Soft agar assays

A 3% solution of agar (at 56°C) was diluted to a final concentration of 0.6% with growth medium (at 56°C), pipetted into tissue culture dishes

and allowed to solidify at room temperature for 20–30 min. At this time, 2×10⁵ cells in a volume of 50 µl were mixed with 0.3% agar (diluted with growth medium at 40°C), pipetted gently onto the bottom agar layer and allowed to solidify for 20–25 min at room temperature. Once solidified, the plates were incubated at 37°C in a 5% CO₂ atmosphere. Fresh top agar was added once a week. After 4 weeks the plates were stained with neutral red.

In vivo tumor growth

Animal experiments in 4- to 6-week-old male athymic Balb/c nu/nu mice were carried out in accordance with both institutional and federal animal care regulations. NIH 3T3 cells expressing the kinase-inactive (K162M) or activated kinase (T288D) construct were grown in DMEM supplemented with 10% FBS. Cells were harvested by trypsinization, centrifuged at 300 g for 5 min, washed twice and resuspended in sterile PBS. 1×10⁶ cells in 0.2 ml were injected subcutaneously between the scapula of each mouse. Tumor volumes were estimated by caliper measurements at 4, 6 and 8 weeks.

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References

- Andreassen,P.R., Palmer,D.K., Wener,M.H. and Margolis,R.L. (1991) Telophase disc: a new mammalian mitotic organelle that bisects cell with a possible function in cytokinesis. *J. Cell Sci.*, **99**, 523–534.
- Beck,T.W., Huleihel,M., Gunnell,M., Bonner,T.I. and Rapp,U.R. (1987) The complete coding sequence of the human A-raf-1 oncogene and transforming activity of a human A-raf carrying retrovirus. *Nucleic Acids Res.*, **15**, 595–609.
- Bellacosa,A., Franke,T.F., Gonzalez-Portal,M.E., Datta,K., Taguchi,T., Gardner,J., Cheng,J.Q., Testa,J.R. and Tsichlis,P.N. (1993) Structure, expression and chromosomal mapping of c-akt: relationship to v-akt and its implications. *Oncogene*, **8**, 745–754.
- Bigner,S.H., Mark,J., Friedman,H.S., Biegel,J.A. and Bigner,D.D. (1988) Structural chromosomal abnormalities in human medulloblastoma. *Cancer Genet. Cytogenet.*, **30**, 91–101.
- Bischoff,J.R., Friedman,P.N., Marshak,D.R., Prives,C. and Beach,D. (1990) Human p53 is phosphorylated by cyclin B-cdc2 and p60-cdc2. *Proc. Natl Acad. Sci. USA*, **87**, 4766–4770.
- Bockmuhl,U., Petersen,I., Schwendel,A. and Dietel,M. (1996) Genetic screening of head-neck carcinomas using comparative genomic hybridization (CGH). *Laryngorhinotologie*, **75**, 408–414.
- Boveri,T. (1929) *The Origin of Malignant Tumors*. Williams & Wilkins, Baltimore, MD.
- Brinkmann,U., Gallo,M., Polymeropoulos,M.H. and Pastan,I. (1996) The human CAS (cellular apoptosis susceptibility) gene mapping on chromosome 20q13 is amplified in BT474 breast cancer cells and part of aberrant chromosomes in breast and colon cancer cell lines. *Genome Res.*, **6**, 187–194.
- Chomczynski,P. and Sacchi,N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, **162**, 156–159.
- Courjal,F., Cuny,M., Rodriguez,C., Louason,G., Speiser,P., Katsaros,D., Tanner,M.M., Zeillinger,R. and Theillet,C. (1996) DNA amplifications at 20q13 and MDM2 define distinct subsets of evolved breast and ovarian tumours. *Br. J. Cancer*, **74**, 1984–1989.
- Cowley,S., Paterson,H., Kemp,P. and Marshall,C.J. (1994) Activation of MAP kinase is necessary and sufficient for PC12 differentiation and for transformation of NIH 3T3 cells. *Cell*, **77**, 841–852.
- el-Deiry,W.S. *et al.* (1993) WAF1, a potential mediator of p53 tumor suppression. *Cell*, **75**, 817–825.
- Elledge,S.J. (1996) Cell cycle checkpoints: preventing an identity crisis. *Science*, **274**, 1664–1672.
- Francisco,L., Wang,W. and Chan,C.S. (1994) Type 1 protein phosphatase acts in opposition to Ipl1 protein kinase in regulating yeast chromosome segregation. *Mol. Cell. Biol.*, **14**, 4731–4740.

- Glover,D.M., Leibowitz,M.H., McLean,D.A. and Parry,H. (1995) Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. *Cell*, **81**, 95–105.
- Golsteyn,R.M., Schultz,S.J., Bartek,J., Ziemiecki,A., Ried,T. and Nigg,E.A. (1994) Cell cycle analysis and chromosomal localization of human Plk1, a putative homologue of the mitotic kinases *Drosophila* polo and *Saccharomyces cerevisiae* Cdc5. *J. Cell Sci.*, **107**, 1509–1517.
- Gopalan,G., Chan,C.S.M. and Donovan,P.J. (1997) A novel mammalian, mitotic spindle-associated kinase is related to yeast and fly chromosome segregation regulators. *J. Cell Biol.*, **138**, 643–656.
- Hartwell,L.H. and Kastan,M.B. (1994) Cell cycle control and cancer. *Science*, **266**, 1821–1828.
- Hoyt,M.A., Totis,L. and Roberts,B.T. (1991) *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell*, **66**, 507–517.
- Hunter,T. (1997) Oncoprotein networks. *Cell*, **88**, 333–346.
- Hunter,T. and Pines,J. (1994) Cyclins and cancer. II: Cyclin D and CDK inhibitors come of age. *Cell*, **79**, 573–582.
- Isola,J.J., Kallioniemi,O.P., Chu,L.W., Fuqua,S.A., Hilsenbeck,S.G., Osborne,C.K. and Waldman,F.M. (1995) Genetic aberrations detected by comparative genomic hybridization predict outcome in node-negative breast cancer. *Am. J. Pathol.*, **147**, 905–911.
- Iwabuchi,H., Sakamoto,M., Sakunaga,H., Ma,Y.Y., Carcangiu,M.L., Pinkel,D., Yang-Feng,T.L. and Gray,J.W. (1995) Genetic analysis of benign, low-grade and high-grade ovarian tumors. *Cancer Res.*, **55**, 6172–6180.
- James,L.A., Mitchell,E.L., Menasce,L. and Varley,J.M. (1997) Comparative genomic hybridisation of ductal carcinoma *in situ* of the breast: identification of regions of DNA amplification and deletion in common with invasive breast carcinoma. *Oncogene*, **14**, 1059–1065.
- Kallioniemi,A., Kallioniemi,O.P., Sudar,D., Rutovitz,D., Gray,J.W., Waldman,F. and Pinkel,D. (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science*, **258**, 818–821.
- Kallioniemi,A. *et al.* (1994) Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. *Proc. Natl Acad. Sci. USA*, **91**, 2156–2160.
- Kimura,M., Kotani,S., Hattori,T., Sumi,N., Yoshioka,T., Todokoro,K. and Okano,Y. (1997) Cell cycle-dependent expression and spindle pole localization of a novel human protein kinase, Aik, related to Aurora of *Drosophila* and yeast Ipl1. *J. Biol. Chem.*, **272**, 13766–13771.
- Kinzel,K.W. and Vogelstein,B. (1997) Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature*, **386**, 761–763.
- Kutay,U., Bischoff,F., Kostka,S., Kraft,R. and Görlich,D. (1997) Export of importin alpha from the nucleus is mediated by a specific nuclear transport factor. *Cell*, **90**, 1061–1071.
- Lane,H.A. and Nigg,E.A. (1996) Antibody microinjection reveals an essential role for human polo-like kinase 1 (Plk1) in the functional maturation of mitotic centrosomes. *J. Cell Biol.*, **135**, 1701–1713.
- Lane,H.A. and Nigg,E.A. (1997) POLO-like kinases join the outer circle. *Trends Cell Biol.*, **7**, 63–68.
- Larramendy,M.L., Tarkkanen,M., Valle,J., Kivioja,A.H., Ervasti,H., Karaharju,E., Salmivalli,T., Elomaa,I. and Knuutila,S. (1997) Gains, losses and amplifications of DNA sequences evaluated by comparative genomic hybridization in chondrosarcomas. *Am. J. Pathol.*, **150**, 685–691.
- Markowitz,D., Goff,S. and Bank,A. (1988) Construction and use of a safe and efficient amphotropic packaging cell line. *Virology*, **167**, 400–406.
- Mitelman,F., Mertens,F. and Johansson,B. (1997) A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. *Nature Genet.*, **15**, 417–474.
- Miyoshi,J., Higashi,T., Mukai,H., Ohuchi,T. and Kakunaga,T. (1991) Structure and transforming potential of the human *cot* oncogene encoding a putative protein kinase. *Mol. Cell. Biol.*, **11**, 4088–4096.
- Morgan,D.O. (1995) Principles of CDK regulation. *Nature*, **374**, 131–134.
- Mossie,K., Jallal,B., Alves,F., Sures,I., Plowman,G.D. and Ullrich,A. (1995) Colon carcinoma kinase-4 defines a new subclass of the receptor tyrosine kinase family. *Oncogene*, **11**, 2179–2184.
- Motokura,T., Bloom,T., Kim,H.G., Juppner,H., Ruderman,J.V., Kronenberg,H.M. and Arnold,A. (1991) A novel cyclin encoded by a *bcl1*-linked candidate oncogene. *Nature*, **350**, 512–515.
- Muleris,M., Salmon,R.J., Dutrillaux,A.M., Vielh,P., Zafrani,B., Girodet,J. and Dutrillaux,B. (1987) Characteristic chromosomal imbalances in 18 near-diploid colorectal tumors. *Cancer Genet. Cytogenet.*, **29**, 289–301.
- Nigg,E.A., Blangy,A. and Lane,H.A. (1996) Dynamic changes in nuclear architecture during mitosis: on the role of protein phosphorylation in spindle assembly and chromosome segregation. *Exp. Cell Res.*, **229**, 174–180.
- Pati,U.K. (1992) Novel vectors for expression of cDNA encoding epitope-tagged proteins in mammalian cells. *Gene*, **114**, 285–288.
- Peles,E., Nativ,M., Lustig,M., Grumet,M., Schilling,J., Martinez,R., Plowman,G.D. and Schlessinger,J. (1997) Identification of a novel contactin-associated transmembrane receptor with multiple domains implicated in protein–protein interactions. *EMBO J.*, **16**, 978–988.
- Reznikoff,C.A., Belair,C.D., Yeager,T.R., Savelieva,E., Blleloch,R.H., Puthenveetil,J.A. and Cuthill,S. (1996) A molecular genetic model of human bladder cancer pathogenesis. *Semin. Oncol.*, **23**, 571–584.
- Savelieva,E., Belair,C.D., Newton,M.A., DeVries,S., Gray,J.W., Waldman,F. and Reznikoff,C.A. (1997) 20q gain associates with immortalization: 20q13.2 amplification correlates with genome instability in human papillomavirus 16 E7 transformed human uroepithelial cells. *Oncogene*, **14**, 551–560.
- Schlegel,J., Stumm,G., Scherthan,H., Bocker,T., Zirngibl,H., Ruschoff,J. and Hofstadter,F. (1995) Comparative genomic *in situ* hybridization of colon carcinomas with replication error. *Cancer Res.*, **55**, 6002–6005.
- Selten,G., Cuyppers,H.T., Boelens,W., Robanus-Maandag,E., Verbeek,J., Domen,J., van Beveren,C. and Berns,A. (1986) The primary structure of the putative oncogene *pim-1* shows extensive homology with protein kinases. *Cell*, **46**, 603–611.
- Sen,S., Zhou,H. and White,R.A. (1997) A putative serine/threonine kinase encoding gene BTAK on chromosome 20q13 is amplified and overexpressed in human breast cancer cell lines. *Oncogene*, **14**, 2195–2200.
- Sherman,F., Fink,G. and Laurence,C. (1974). *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Sherr,C.J. (1994) G₁ phase progression: cycling on cue. *Cell*, **79**, 551–555.
- Sherr,C.J. (1996) Cancer cell cycles. *Science*, **274**, 1672–1677.
- Sikorski,R.S. and Hieter,P. (1989) A system of shuttle vectors and yeast host strain designed or efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics*, **122**, 19–27.
- Slamon,D.J., Clark,G.M., Wong,S.G., Levin,W.J., Ullrich,A. and McGuire,W.L. (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*, **235**, 177–182.
- Smith,M.R., Wilson,M.L., Hamaoka,R., Chase,D., Kung,H., Longo,D.L. and Ferris,D.K. (1997) Malignant transformation of mammalian cells initiated by constitutive expression of the polo-like kinase. *Biochem. Biophys. Res. Commun.*, **234**, 397–405.
- Solinas-Toldo,S., Wallrapp,C., Muller-Pillasch,F., Bentz,M., Gress,T. and Lichter,P. (1996) Mapping of chromosomal imbalances in pancreatic carcinoma by comparative genomic hybridization. *Cancer Res.*, **56**, 3803–3807.
- Tang,Y., Chen,Z., Ambrose,D., Liu,J., Gibbs,J.B., Chernoff,J. and Field,J. (1997) Kinase-deficient Pak 1 mutants inhibit ras transformation of Rat-1 fibroblasts. *Mol. Cell. Biol.*, **17**, 4454–4464.
- Tanner,M.M. *et al.* (1994) Increased copy number at 20q13 in breast cancer: defining the critical region and exclusion of candidate genes. *Cancer Res.*, **54**, 4257–4260.
- Tanner,M.M. *et al.* (1996) Independent amplification and frequent co-amplification of three nonsyntenic regions on the long arm of chromosome 20 in human breast cancer. *Cancer Res.*, **56**, 3441–3445.
- Taylor,S.S. and McKeon,F. (1997) Kinetochores localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. *Cell*, **89**, 727–735.
- Terada,Y., Tatsuka,M., Suzuki,F., Yasudo,Y., Fujita,S. and Otsu,M. (1998) AIM-1: a mammalian midbody-associated protein required for cytokinesis. *EMBO J.*, **17**, 677–676.
- Tirkkonen,M. *et al.* (1997) Distinct somatic genetic changes associated with tumor progression in carriers of BRCA1 and BRCA2 germ-line mutations. *Cancer Res.*, **57**, 1222–1227.
- Van Beveren,C., Galleshaw,J.A., Jonas,V., Berns,A.J., Doolittle,R.F., Donoghue,D.J. and Verma,I.M. (1981) Nucleotide sequence and formation of the transforming gene of a mouse sarcoma virus. *Nature*, **289**, 258–262.
- van de Vijver,M.J. (1993) Molecular genetic changes in human breast cancer. *Adv. Cancer Res.*, **61**, 25–56.
- Weiss,E. and Winey,M. (1996) The *Saccharomyces cerevisiae* spindle pole body duplication gene Mps1 is part of a mitotic checkpoint. *J. Cell Biol.*, **132**, 111–123.

- Wolf,G., Elez,R., Doermer,A., Holtrich,U., Ackermann,H., Stutte,H.J., Altmannsberger,H.M., Rubsamen-Waigmann,H. and Strebhardt,K. (1997) Prognostic significance of polo-like kinase (PLK) expression in non-small cell lung cancer. *Oncogene*, **14**, 543–549.
- Xiong,Y., Hannon,G.J., Zhang,H., Casso,D., Kobayashi,R. and Beach,D. (1993) p21 is a universal inhibitor of cyclin kinases. *Nature*, **366**, 701–704.
- Yanai,A., Arama,E., Kilfin,G. and Motro,B. (1997) Ayk1, a novel mammalian gene related to *Drosophila* aurora centrosome separation kinase, is specifically expressed during meiosis. *Oncogene*, **14**, 2943–2950.
- Yaseen,N.Y., Watmore,A.E., Potter,A.M., Potter,C.W., Jacob,G. and Rees,R.C. (1990) Chromosome studies in eleven colorectal tumors. *Cancer Genet. Cytogenet.*, **44**, 83–97.
- Zhu,Y., Peterson,C.L. and Christman,M.F. (1995) HPR1 encodes a global positive regulator of transcription in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **15**, 1698–1708.

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