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(54) Title: CELL-CYCLE CHECKPOINT GENES

(57) Abstract

This invention relates to a class of checkpoint genes and their polypeptide products which control progression through the cell cycle in eukaryotic cells. In particular this invention relates to *Schizosaccharomyces pombe rad3* gene, to its human homologue (ATR) and to their encoded proteins. The invention further relates to assay methods for selecting compounds which modulate the activity of the polypeptide products of these checkpoint genes and the use of the selected compounds in anticancer therapy.

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Cell-cycle checkpoint genes

The present invention relates to a class of checkpoint genes which control progression through the cell cycle in eukaryotic cells.

Background to the invention.

Control of the cell cycle is fundamental to the growth and maintenance of eukaryotic organisms, from yeasts to mammals. Eukaryotic cells have evolved control pathways, termed "checkpoints" which ensure that individual steps of the cell cycle are completed before the next step occurs. In response to DNA damage, cell survival is increased both by direct DNA repair mechanisms and by delaying progression through the cell cycle. Depending on the position of the cell within the cycle at the time of irradiation, DNA damage in mammalian cells can prevent (a) passage from G1 into S phase, (b) progression through S phase or (c) passage from G2 into mitosis. Such checkpoints are thought to prevent deleterious events such as replication of damaged DNA and the segregation of fragmented chromosomes during mitosis (Hartwell and Kastan, 1994).

The *rad3* gene of *Schizosaccharomyces pombe* is required for the checkpoints that respond to DNA damage and replication blocks. Rad3 is a member of the lipid kinase subclass of kinases which possess regions having sequence homology to the lipid kinase domain of the p110 subunit of phosphatidylinositol-3 kinase (PI-3 kinase). This subclass also includes the ATM protein defective in ataxia-telangiectasia patients. Cells from ataxia telangiectasia patients (AT cells) have lost the delay to S phase following irradiation and are said to display radio resistant DNA synthesis (Painter and Young, 1989). AT cells irradiated in S phase accumulate in G2 with lethal damage, presumably as a consequence of attempting to replicate damaged DNA. AT cells irradiated during G2 display a different phenotype: they do not arrest mitosis after DNA damage, and progress through mitosis with damaged DNA (Beamish and Lavin, 1994). Mutations at the A-T locus, to which the *ATM* gene has been mapped, thus result in disruption of several checkpoints required for an appropriate response to ionising radiation. Other members of this lipid kinase subclass include: Tel1p (Greenwell *et al.* 1985), a gene involved in maintaining proper telomere length in *Saccharomyces*

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cerevisiae; Esr1p; Mec1p and the product of the *Drosophila melanogaster mei-41* checkpoint gene (Hari *et al.* 1995).

Disclosure of the invention.

We have analyzed the *S. pombe rad3* gene and found that it has a full length amino acid sequence of 2386 amino acids, not the 1070 amino acids described by Seaton *et al.* 1992. We have determined that this is the direct homologue of *S. cerevisiae* Esr1p, and that it shares the same overall structure as the ATM gene. The C-terminal region of the *rad3* protein contains a lipid kinase domain, which is required for Rad3 function. We have shown that Rad3 is capable of self association. We have also identified a protein kinase activity associated with Rad3.

Further, we have found a human homologue to *rad3*. This gene, which we have named ATR (ataxia and rad related), displays significantly higher homology to *rad3* than it does to the ATM gene.

The human ATR cDNA sequence is set out as Seq. ID No. 1. The amino acid sequence of the ORF from nucleotides 80 and 8011 is set out as Seq. ID No. 2.

The DNA sequence of the open reading frame (ORF) of *rad3* is shown as Seq. ID. No. 3. The 2386 amino acid translation of the gene (nucleotides 585 to 7742 of Seq. ID No. 3) is shown as Seq. ID. No. 4.

Accordingly, in a first aspect, the invention provides the ATR protein of Seq. ID. 2 and homologues thereof, polypeptide fragments thereof, as well as antibodies capable of binding the ATR protein or polypeptide fragments thereof. ATR proteins, homologues and fragments thereof are referred to below as polypeptides of the invention.

In another aspect, the present invention provides a polynucleotide in substantially isolated form capable of hybridising selectively to Seq.ID No 1 or to the complement (i.e. opposite strand) thereof. Also provided are polynucleotides encoding polypeptides of the invention.

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Such polynucleotides will be referred to as a polynucleotide of the invention. A polynucleotides of the invention includes DNA of Seq.ID Nos 1 and fragments thereof capable of selectively hybridising to this gene.

In a further aspect, the invention provides recombinant vectors carrying a polynucleotide of the invention, including expression vectors, and methods of growing such vectors in a suitable host cell, for example under conditions in which expression of a protein or polypeptide encoded by a sequence of the invention occurs.

In an additional aspect, the invention provides kits comprising polynucleotides, polypeptides or antibodies of the invention and methods of using such kits in diagnosing the presence of absence of ATR and its homologues, or variants thereof, including deleterious ATR mutants.

The invention further provides assay methods for screening candidate substances for use as compounds for inhibiting or activating ATR activity, or the activity of mutated forms of ATR which are deficient in checkpoint activity. The invention also provides assay methods for screening candidate substances for use as compounds for inhibiting interactions between ATR and other compounds that interact with ATR, including ATR itself.

In a related aspect, the invention also provides a polynucleotide sequence of Seq. ID No. 3 in substantially isolated form, and the protein of Seq. ID No. 4 in substantially isolated form, and novel fragments and variants thereof.

Detailed description of the invention.

A. Polynucleotides.

Polynucleotides of the invention may comprise DNA or RNA. They may also be polynucleotides which include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the present invention, it is to be understood that the polynucleotides described herein may be modified by any method

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