(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 February 2007 (08.02.2007)

PCT

(10) International Publication Number
WO 2007/015840 A2

(51) International Patent Classification:
G01N 33/574 (2006.01)

(21) International Application Number:
PCT/US2006/027791

(22) International Filing Date: 18 July 2006 (18.07.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/700,979	20 July 2005 (20.07.2005)	US
11/487,883	17 July 2006 (17.07.2006)	US

(71) Applicant *(for all designated States except US)*: **CELL SIGNALING TECHNOLOGY, INC.** [US/US]; 3 Trask Lane, Danvers, MA 01923 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LIVINGSTONE, Mark** [US/CA]; 33 Cote Ste. Catherine, Apt. 2411, Montreal, QC H2V2A1 (CA). **RUAN, Hong** [US/US]; 15 Beacon Drive, Middleton, MA 01949 (US). **POLAKIEWICZ, Roberto** [US/US]; 4 Lincoln Terrace, Lexington, MA 02421 (US).

(74) Agent: **CULLEM, James, Gregory**; CELL SIGNALING TECHNOLOGY, INC., Intellectual Property Counsel, 3 Trask Lane, Danvers, MA 01923 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

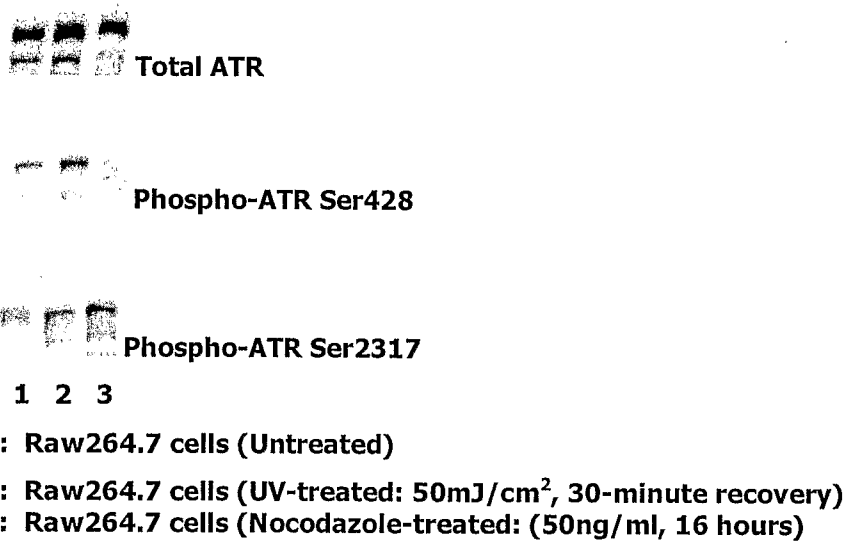
— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report*

[Continued on next page]

(54) Title: REAGENTS FOR THE DETECTION OF PHOSPHORYLATED ATR KINASE (SER 428/SER 2317) AND USES THEREOF



(57) Abstract: The invention discloses two novel phosphorylation sites in human ATR kinase, serine 428 (Ser428) and serine 2317 (Ser2317) respectively, and provides reagents, including antibodies and AQUA peptides, that selectively bind to and/or detect ATR only when phosphorylated at one or more of these respective sites, but do not bind to ATR when not phosphorylated at these respective sites. Also provided are methods for determining the phosphorylation of ATR kinase in a biological sample, by using a detectable reagent that binds to ATR only when phosphorylated at Ser428 and/or Ser2317. Kits comprising the ATR (Ser428, Ser2317)- specific reagents of the invention are also provided.

WO 2007/015840 A2

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

REAGENTS FOR THE DETECTION OF PHOSPHORYLATED ATR KINASE (SER 428/SER 2317) AND USES THEREOF

RELATED APPLICATIONS

This application claims priority to, and the benefit of, U.S.S.N. 60/700,979, filed July 20, 2005 (presently pending), the disclosure of which is hereby incorporated by reference.

FIELD OF THE INVENTION

The invention relates generally to antibodies and peptide reagents for the detection of protein phosphorylation, and to protein phosphorylation in cancer.

BACKGROUND OF THE INVENTION

The activation of proteins by post-translational modification is an important cellular mechanism for regulating most aspects of biological organization and control, including growth, development, homeostasis, and cellular communication. Protein phosphorylation, for example, plays a critical role in the etiology of many pathological conditions and diseases, including cancer, developmental disorders, autoimmune diseases, and diabetes. Yet, in spite of the importance of protein modification, it is not yet well understood at the molecular level, due to the extraordinary complexity of signaling pathways, and the slow development of technology necessary to unravel it.

Protein phosphorylation on a proteome-wide scale is extremely complex as a result of three factors: the large number of modifying proteins, *e.g.* kinases, encoded in the genome, the much larger number of sites on substrate proteins that are modified by these enzymes, and the

dynamic nature of protein expression during growth, development, disease states, and aging. The human genome, for example, encodes over 520 different protein kinases, making them the most abundant class of enzymes known. *See* Hunter, *Nature 411:* 355-65 (2001). Most kinases phosphorylate many different substrate proteins, at distinct tyrosine, serine, and/or threonine residues. Indeed, it is estimated that one-third of all proteins encoded by the human genome are phosphorylated, and many are phosphorylated at multiple sites by different kinases.

Many of these phosphorylation sites regulate critical biological processes and may prove to be important diagnostic or therapeutic targets for molecular medicine. For example, of the more than 100 dominant oncogenes identified to date, 46 are protein kinases. *See* Hunter, *supra*. Understanding which proteins are modified by these kinases will greatly expand our understanding of the molecular mechanisms underlying oncogenic transformation. Therefore, the identification of, and ability to detect, phosphorylation sites on a wide variety of cellular proteins is crucially important to understanding the key signaling proteins and pathways implicated in the progression of diseases like cancer.

Deregulation of kinases is a central theme in the etiology of cancers. Constitutively active kinases can contribute not only to unrestricted cell proliferation, but also to other important features of malignant tumors, such as evading apoptosis, the ability to promote blood vessel growth, the ability to invade other tissues and build metastases at distant sites (*see e.g.* Blume-Jensen *et al.*, *Nature 411:* 355-365 (2001)). These effects are mediated not only through aberrant activity of receptor kinase themselves, but, in turn, by aberrant activity of their downstream signaling molecules and substrates, including kinases.

Among such kinases is ataxia-telangiectasia and Rad3-related (ATR) kinase, a serine/threonine protein kinase that is implicated in cellular DNA damage repair processes and cell cycle signaling. Mutations of ATR have been linked to cancers of the stomach and endometrium, and lead to increased sensitivity to ionizing radiation and abolished cell cycle checkpoints. ATR is essential for the viability of somatic cells, and deletion of ATR has been shown to result in loss of damage checkpoint responses and cell death. *See* Cortez *et al.*, *Science 294:* 1713-1716 (2001). ATR is also essential for the stability of fragile sites, and low ATR expression in Seckel syndrome patients results in increased chromosomal breakage following replication stress. *See* Casper *et al.*, *Am. J. Hum. Genet. 75:* 654-660 (2004). The replication protein A (RPA) complex recruits ATR, and its interacting protein ATRIP, to sites of DNA damage, and ATR itself mediates the activation of the CHK1 signaling cascade. *See* Zou *et al.*, *Science 300:* 1542-1548 (2003). ATR, like its related checkpoint kinase ATM, phosphorylates RAD17 early in a cascade that is critical to for checkpoint signaling in DNA-damaged cells. *See* Bao *et al.*, *Nature 411:* 969-974 (2001). It is believed that ATR is particularly essential in the early mammalian embryo, to sense incomplete DNA replication and prevent mitotic catastrophe.

Despite the essential role of ATR in cell cycle signaling and DNA repair processes, little is known about its activation, and there are no known phosphorylation sites on this protein. Since kinase activity is regulated through phosphorylation, there remains a need for identifying phosphorylation sites on ATR, and for subsequently developing novel reagents to study the phosphorylation of ATR at such sites. Identifying particular phosphorylation sites on ATR and providing new reagents to detect and quantify them remains especially important to advancing our

understanding of the regulation of ATR and the role it plays in cell cycling, DNA repair, and disease.

SUMMARY OF THE INVENTION

The invention discloses two novel human ATR kinase phosphorylation sites, serine 428 (Ser428) and serine 2317 (Ser2317), as well as homologous sites other mammals, and provides antibodies and AQUA peptides that selectively bind to and/or detect ATR when phosphorylated at these sites. Also provided are methods for determining the phosphorylation of ATR in a biological sample, profiling ATR activation in a test tissue, and identifying a compound that modulates expression and/or activity of ATR, by using a detectable reagent, such as the disclosed antibodies or AQUA peptides, that selectively binds to and or quantifies ATR when phosphorylated at Ser428 and/or Ser2317, respectively.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1– is the amino acid sequence (1-letter code) of human ATR kinase (SEQ ID NO: 1) (SwissProt Acc# Q13535). Ser428 and Ser2317 are underlined, and the peptide sequences encompassing Ser428 and Ser2317, respectively, and corresponding to the immunogen used to generate exemplary ATR(Ser428) and ATR(Ser2317) phosphospecific antibodies are indicated in bold (*see* Example 1).

Fig. 2– is the amino acid sequence (1-letter code) of mouse ATR (SEQ ID NO: 2) (SwissProt Acc# Q9JKK8). Ser419 and Ser2296 are underlined, and the peptide sequences encompassing Ser419 and Ser2296, respectively, corresponding to the highly homologous sequences of the human ATR (Ser428, Ser2317) phosphorylation sites are indicated in bold (*see* Fig. 1).

Fig. 3 – shows Western blot analyses using phospho-ATR(Ser428) and phospho-ATR(Ser2317) polyclonal antibodies and 264.7 cells, treated with either Nocodazole or ultraviolet radiation (UV).

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, two novel sites of ATR kinase phosphorylation, serine 428 (Ser428) and serine 2317 (Ser2317) in the human sequence (*see* Figure 1), have now been identified. Highly homologous novel phosphorylation sites in murine ATR, serine 419 (Ser419) and serine 2296 (Ser2296) (*see* Figure 2), have also been identified. Although ATR kinase plays a critical role in DNA damage repair and cell cycle checkpoint signaling, the presently disclosed phosphorylation sites were previously been unknown.

The ATR Ser428 and Ser2317 phosphorylation sites were identified/predicted by analyzing the human ATR amino acid sequence with the ScanSite program (http://scansite.mit.edu) (*see also* Yaffe *et al.*, *Nat Biotechnol. 19(4):* 348-53 (2001)). This algorithm searches for motifs within proteins that are likely to be phosphorylated by specific protein kinases or bind to domains such as SH2 domains, 14-3-3 domains or PDZ domains. Optimal phosphorylation sites for particular serine/threonine protein kinases or tyrosine protein kinases are predicted using a matrix of selectivity values for amino acids at each position relative to the phosphorylation site, as determined from the oriented peptide library technique described by Songyang *et al.*, Current Biology 4: 973-982 (1994) and Songyang *et al.*, Nature 373: 536-539 (1995).

Analysis of the human ATR kinase protein sequence with ScanSite with high stringency revealed five (5) potential Ser/Thr phosphorylation sites, including putative Cdc2, Cdk5, Casein Kinase 1 and PKC zeta, PKC mu and DNA-PK sites. Attention was focused on the Cdc2/Cdk5 and

PKC zeta sites. The two sites identified, numbered according to human ATR (Figure 1, SEQ ID NO: 1), all include a typical PKC consensus (KXX*SXXK) motif or a proline directed kinase motif sequence, and are (phosphorylated serine indicated by bold *S):

Ser428: SSNSDGI***S**PKRRRLS (SEQ ID NO: 3)
Ser2317: KKISLKG*SDGKFYIM (SEQ ID NO: 4)

Phosphorylation of human ATR at Ser428 and Ser2317 was confirmed using exemplary phospho-specific antibodies of the invention (*see* Examples). As a result of this discovery, reagents may now be produced that only detect ATR kinase when phosphorylated at either of these sites. For example, peptide antigens may now be designed to raise phospho-specific antibodies that bind ATR only when phosphorylated at Ser428 or Ser2317 in the human ATR sequence, and/or to the equivalent and highly homologous sites in mouse ATR (Ser419, Ser2296), or other species, such as Xenopus laevis (Ser2328).

The discovery of novel ATR kinase phosphorylation sites described herein enables the production, by standard methods, of new reagents, such as phosphorylation site-specific antibodies and AQUA peptides (heavy-isotope labeled peptides), capable of specifically detecting and/or quantifying these phosphorylated sites/proteins. Such reagents are highly useful, *inter alia*, for studying signal transduction events underlying the progression of diseases, like cancer, involving aberrant DNA damage repair and cell cycle checkpoint signaling. Accordingly, the invention provides novel reagents -- phospho-specific antibodies and AQUA peptides -- for the specific detection and/or quantification of ATR kinase only when phosphorylated (or only when not phosphorylated) at a particular phosphorylation site disclosed herein. The invention also provides methods of detecting and/or quantifying phosphorylated ATR using the phosphorylation-site specific antibodies and AQUA peptides of the invention.

In part, the invention provides an isolated phosphorylation site-specific antibody that specifically binds human ATR kinase only when phosphorylated at serine 428 or serine 2317, respectively, and does not bind the unphosphorylated form of the protein and/or other phosphorylation sites on ATR. In further part, the invention provides a heavy-isotope labeled peptide (AQUA peptide) for the detection and quantification of human ATR kinase, the labeled peptide comprising a phosphorylation site sequence encompassing either serine 428 or serine 2317 in the human ATR sequence (see Figure 1). Preferred AQUA peptides of the invention are tryptic digest fragments that encompass either of the novel phosphorylation sites disclosed herein, as further described below. Antibodies and AQUA peptides for detecting highly homologous phosphorylation sites in other species, such as mouse and Xenopus, as discussed above, are within the scope of the present invention.

Also provided are methods of using a detectable reagent that binds to phosphorylated ATR (Ser428 and Ser2317) to detect ATR phosphorylation and activation in a biological sample or test tissue potentially containing, or suspected of containing, phosphorylated ATR, or having altered ATR expression or activity, as further described below. In a preferred embodiment, the detectable reagent is at least one ATR (Ser428, Ser2317) antibody of the invention, and the sample or tissue is taken from a subject potentially having, or suspected of having, altered ATR activity. In another preferred embodiment, the detectable reagent is an AQUA peptide of the invention.

The further aspects, advantages, and embodiments of the invention are described in more detail below. All references cited herein are hereby incorporated by reference.

A. Antibodies and Cell Lines

ATR phospho-specific antibodies of the invention bind to human ATR only when phosphorylated at Ser428 or Ser2317, respectively, and do not substantially bind to ATR when not phosphorylated at either these respective residues, nor to ATR when phosphorylated at other phosphorylation sites. The ATR antibodies may also bind highly homologous and equivalent ATR sites in other species, for example mouse ATR (Ser419) and/or ATR (Ser2296), respectively, as disclosed herein.

ATR antibodies of the invention include (a) monoclonal antibodies that bind phospho-ATR (Ser428) or phospho-ATR (Ser2317), (b) polyclonal antibodies which bind to phospho-ATR (Ser428) and/or phospho-ATR (Ser2317), (c) antibodies (monoclonal or polyclonal) which specifically bind to the phospho-antigen (or more preferably the epitope) bound by the exemplary ATR (Ser428, Ser2317) antibodies disclosed in the Examples herein, (d) antibodies as described in (a)-(c) above that bind equivalent phosphorylation ATR and/or ATR sites in other species (*e.g.* mouse, rat), as disclosed herein, and (e) fragments of (a), (b), (c), or (d) above which bind to the antigen (or more preferably the epitope) bound by the exemplary antibodies disclosed herein. Such antibodies and antibody fragments may be produced by a variety of techniques well known in the art, as discussed below. Antibodies that bind to the phosphorylated epitope (*i.e.*, the specific binding site) bound by the exemplary ATR (Ser428, Ser2317) antibodies of the Examples herein can be identified in accordance with known techniques, such as their ability to compete with labeled ATR antibodies in a competitive binding assay.

The preferred epitopic site of the human ATR (Ser428, Ser2317) antibodies of the invention is a peptide fragment consisting essentially of about 11 to 17 amino acids including the phosphorylated serine 428 or

serine 2317, wherein about 5 to 8 amino acids are positioned on each side of the serine phosphorylation site (for example, residues 427-433 of SEQ ID NO: 1, or residues 2312-2322 of SEQ ID NO: 1). These epitopic sites, for example, correspond to the following equivalent murine sites: residues 414-424 of SEQ ID NO: 2 (mouse ATR) (encompassing Ser419) and residues 2291-2301 of SEQ ID NO: 2 (mouse ATR) encompassing Ser2296).

The invention is not limited to ATR antibodies, but includes equivalent molecules, such as protein binding domains or nucleic acid aptamers, which bind, in a phospho-specific manner, to essentially the same phosphorylated epitope to which the ATR antibodies of the invention bind. *See, e.g.*, Neuberger *et al., Nature 312:* 604 (1984). Such equivalent non-antibody reagents may be suitably employed in the methods of the invention further described below.

The term "antibody" or "antibodies" as used herein refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE. The antibodies may be monoclonal or polyclonal and may be of any species of origin, including (for example) mouse, rat, rabbit, horse, or human, or may be chimeric antibodies. *See, e.g.*, M. Walker *et al.*, *Molec. Immunol. 26:* 403-11 (1989); Morrision *et al.*, *Proc. Nat'l. Acad. Sci. 81:* 6851 (1984); Neuberger *et al.*, *Nature 312:* 604 (1984)). The antibodies may be recombinant monoclonal antibodies produced according to the methods disclosed in U.S. Pat. No. 4,474,893 (Reading) or U.S. Pat. No. 4,816,567 (Cabilly *et al.*) The antibodies may also be chemically constructed specific antibodies made according to the method disclosed in U.S. Pat. No. 4,676,980 (Segel *et al.*)

The term "ATR antibodies" is used interchangeably with the term "ATR (Ser428, Ser2317) antibodies" which means antibodies that specifically bind phospho-ATR (Ser428) or phospho-ATR (Ser2317) (in the human sequence), both monoclonal and polyclonal, as disclosed

herein. The term includes antibodies that bind equivalent and highly-homologous sites in ATR from other species, for example, murine ATR (Ser419, Ser2296), Xenopus laevis ATR (Ser2328), etc. The term "does not bind" with respect to disclosed antibodies means does not substantially react with as compared to binding to phospho-ATR and/or phospho-ATR. The term includes antibodies that bind whole protein comprising the target phosphorylation site, as well as shorter ATR polypeptides or fragments comprising the phosphorylated serine residue (*e.g.* a polypeptide of 5-25 or 25-50 or more residues comprising the target phosphorylation site).

The term "detectable reagent" means a molecule, including an antibody, peptide fragment, binding protein domain, etc., the binding of which to a desired target is detectable or traceable. Suitable means of detection are described below.

Polyclonal antibodies of the invention may be produced according to standard techniques by immunizing a suitable animal (*e.g.*, rabbit, goat, etc.) with an antigen encompassing phospho-Ser428 or phospho-Ser2317 (human ATR sequence), collecting immune serum from the animal, and separating the polyclonal antibodies from the immune serum, in accordance with known procedures. In a preferred embodiment, the antigen is a phospho-peptide antigen comprising the human ATR sequence surrounding and including phospho-Ser428 or phospho-Ser2317, respectively, the antigen being selected and constructed in accordance with well-known techniques. *See, e.g.*, ANTIBODIES: A LABORATORY MANUAL, Chapter 5, p. 75-76, Harlow & Lane Eds., Cold Spring Harbor Laboratory (1988); Czernik, *Methods In Enzymology, 201:* 264-283 (1991); Merrifield, *J. Am. Chem. Soc. 85:* 21-49 (1962)).

Particularly preferred peptide antigens, SNSDGI***S**PKRRRL (SEQ ID NO: 5), and ISLKG***S**DGKFY (SEQ ID NO: 6) (where ***S** = phosphoserine) (corresponding to the ATR Ser428 and Ser2317

phosphorylation sites, respectively (see Figure 1) are described in Example 1, below. It will be appreciated by those of skill in the art that longer or shorter phosphopeptide antigens may be employed. *See Id.* Polyclonal ATR antibodies produced as described herein may be screened as further described below. These preferred antigens corresponds to the equivalent phosphorylation sites in murine ATR (*see* Figure 2).

Monoclonal antibodies of the invention may be produced in a hybridoma cell line according to the well-known technique of Kohler and Milstein. *Nature 265:* 495-97 (1975); Kohler and Milstein, *Eur. J. Immunol. 6:* 511 (1976); *see also*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel *et al.* Eds. (1989). Monoclonal antibodies so produced are highly specific, and improve the selectivity and specificity of diagnostic assay methods provided by the invention. For example, a solution containing the appropriate antigen may be injected into a mouse and, after a sufficient time (in keeping with conventional techniques), the mouse sacrificed and spleen cells obtained. The spleen cells are then immortalized by fusing them with myeloma cells, typically in the presence of polyethylene glycol, to produce hybridoma cells. The hybridoma cells are then grown in a suitable selection media, such as hypoxanthine-aminopterin-thymidine (HAT), and the supernatant screened for monoclonal antibodies having the desired specificity, as described below. The secreted antibody may be recovered from tissue culture supernatant by conventional methods such as precipitation, ion exchange or affinity chromatography, or the like.

Monoclonal Fab fragments may also be produced in *Escherichia coli* by recombinant techniques known to those skilled in the art. *See, e.g.,* W. Huse, *Science 246:* 1275-81 (1989); Mullinax *et al.*, *Proc. Nat'l Acad. Sci. 87:* 8095 (1990). If monoclonal antibodies of one isotype are preferred for a particular application, particular isotypes can be prepared

directly, by selecting from the initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of different isotype by using the sib selection technique to isolate class-switch variants (Steplewski, *et al.*, *Proc. Nat'l. Acad. Sci., 82:* 8653 (1985); Spira *et al.*, *J. Immunol. Methods, 74:* 307 (1984)).

The invention also provides hybridoma clones, constructed as described above, that produce ATR monoclonal antibodies of the invention. Similarly, the invention includes recombinant cells producing a phospho-ATR (Ser428, Ser2317) antibody as disclosed herein, which cells may be constructed by well known techniques; for example the antigen combining site of the monoclonal antibody can be cloned by PCR and single-chain antibodies produced as phage-displayed recombinant antibodies or soluble antibodies in *E. coli* (*see, e.g.,* ANTIBODY ENGINEERING PROTOCOLS, 1995, Humana Press, Sudhir Paul editor.)

ATR antibodies of the invention, whether polyclonal or monoclonal, may be screened for epitope and phospho-specificity according to standard techniques. *See, e.g. Czernik et al., Methods in Enzymology, 201:* 264-283 (1991). For example, the antibodies may be screened against the phospho and non-phospho peptide library by ELISA to ensure specificity for both the desired antigen (*i.e.* that epitope including Ser428, Ser2317) and for reactivity only with the phosphorylated form of the antigen. Peptide competition assays may be carried out to confirm lack of reactivity with other ATR phospho-epitopes. The antibodies may also be tested by Western blotting against cell preparations containing ATR, *e.g.* cell lines over-expressing ATR, to confirm reactivity with the desired phosphorylated target. Specificity against the desired phosphorylated epitopes may also be examined by construction ATR mutants lacking phosphorylatable residues at positions outside the desired epitope known to be phosphorylated, or by mutating the desired phospho-epitope and confirming lack of reactivity. ATR antibodies of the invention may exhibit

some limited cross-reactivity with non-ATR epitopes. This is not unexpected as most antibodies exhibit some degree of cross-reactivity, and anti-peptide antibodies will often cross-react with epitopes having high homology to the immunizing peptide. *See, e.g., Czernik, supra.* Cross-reactivity with non-ATR proteins is readily characterized by Western blotting alongside markers of known molecular weight. Amino acid sequences of cross-reacting proteins may be examined to identify sites highly homologous to the human ATR sequence surrounding Ser428, Ser2317.

ATR antibodies may be further characterized via immuno-histochemical (IHC) staining using normal and diseased tissues to examine ATR phosphorylation and activation status in diseased tissue. IHC may be carried out according to well-known techniques. *See, e.g.*, ANTIBODIES: A LABORATORY MANUAL, Chapter 10, Harlow & Lane Eds., Cold Spring Harbor Laboratory (1988). Briefly, paraffin-embedded tissue (*e.g.* tumor tissue) is prepared for immunohistochemical staining by deparaffinizing tissue sections with xylene followed by ethanol; hydrating in water then PBS; unmasking antigen by heating slide in sodium citrate buffer; incubating sections in hydrogen peroxide; blocking in blocking solution; incubating slide in primary antibody and secondary antibody; and finally detecting using ABC avidin/biotin method according to manufacturer's instructions.

ATR antibodies of the invention bind to human ATR when phosphorylated at the Ser428 or Ser2317 site, respectively, but are not limited only to the human species, *per se*. Phospho-specific antibodies that bind conserved and highly homologous phosphorylation sites in other species (*e.g.* mouse, rat, monkey, *Xenopus*, yeast), in addition to binding the human ATR (pSer428, pSer2317) sites, are within the scope of the present invention. For example, ATR antibodies provided may also bind the highly homologous Ser419 and/or Ser2296 sites in mouse ATR,

respectively, as well as the homologous Ser2328 site in *Xenopus laevis* ATR (SwissProt acc. no. Q13535). Additional highly homologous sites conserved in other species, which are in within the scope of the invention, can readily be identified by standard sequence comparisons, such as using BLAST, with the human ATR and mouse ATR sites disclosed herein.

C. Heavy-Isotope Labeled Peptides (AQUA Peptides).

The novel ATR kinase phosphorylation sites disclosed herein now enable the production of corresponding heavy-isotope labeled peptides for the absolute quantification of such signaling proteins (both phosphorylated and not phosphorylated at a disclosed site) in biological samples. The production and use of AQUA peptides for the absolute quantification of proteins (AQUA) in complex mixtures has been described. *See* WO/03016861, "Absolute Quantification of Proteins and Modified Forms Thereof by Multistage Mass Spectrometry," Gygi *et al. and also* Gerber *et al. Proc. Natl. Acad. Sci. U.S.A. 100:* 6940-5 (2003) (the teachings of which are hereby incorporated herein by reference, in their entirety).

The AQUA methodology employs the introduction of a known quantity of at least one heavy-isotope labeled peptide standard (which has a unique signature detectable by LC-SRM chromatography) into a digested biological sample in order to determine, by comparison to the peptide standard, the absolute quantity of a peptide with the same sequence and protein modification in the biological sample. Briefly, the AQUA methodology has two stages: peptide internal standard selection and validation and method development; and implementation using validated peptide internal standards to detect and quantify a target protein in sample. The method is a powerful technique for detecting and quantifying a given peptide/protein within a complex biological mixture,

such as a cell lysate, and may be employed, *e.g.*, to quantify change in protein phosphorylation as a result of drug treatment, or to quantify differences in the level of a protein in different biological states.

Generally, to develop a suitable internal standard, a particular peptide (or modified peptide) within a target protein sequence is chosen based on its amino acid sequence and the particular protease to be used to digest. The peptide is then generated by solid-phase peptide synthesis such that one residue is replaced with that same residue containing stable isotopes (^{13}C, ^{15}N). The result is a peptide that is chemically identical to its native counterpart formed by proteolysis, but is easily distinguishable by MS via a 7-Da mass shift. A newly synthesized AQUA internal standard peptide is then evaluated by LC–MS/MS. This process provides qualitative information about peptide retention by reverse-phase chromatography, ionization efficiency, and fragmentation via collision-induced dissociation. Informative and abundant fragment ions for sets of native and internal standard peptides are chosen and then specifically monitored in rapid succession as a function of chromatographic retention to form a selected reaction monitoring (LC–SRM) method based on the unique profile of the peptide standard.

The second stage of the AQUA strategy is its implementation to measure the amount of a protein or modified protein from complex mixtures. Whole cell lysates are typically fractionated by SDS-PAGE gel electrophoresis, and regions of the gel consistent with protein migration are excised. This process is followed by in-gel proteolysis in the presence of the AQUA peptides and LC–SRM analysis. (*See* Gerber *et al. supra*.) AQUA peptides are spiked in to the complex peptide mixture obtained by digestion of the whole cell lysate with a proteolytic enzyme and subjected to immunoaffinity purification as described above. The retention time and fragmentation pattern of the native peptide formed by digestion (e.g. trypsinization) is identical to that of the AQUA internal standard peptide

determined previously; thus, LC–MS/MS analysis using an SRM experiment results in the highly specific and sensitive measurement of both internal standard and analyte directly from extremely complex peptide mixtures. Because an absolute amount of the AQUA peptide is added (*e.g.* 250 fmol), the ratio of the areas under the curve can be used to determine the precise expression levels of a protein or phosphorylated form of a protein in the original cell lysate. In addition, the internal standard is present during in-gel digestion as native peptides are formed, such that peptide extraction efficiency from gel pieces, absolute losses during sample handling (including vacuum centrifugation), and variability during introduction into the LC–MS system do not affect the determined ratio of native and AQUA peptide abundances.

An AQUA peptide standard is developed for a known phosphorylation site sequence previously identified by the IAP-LC-MS/MS method within a target protein. One AQUA peptide incorporating the phosphorylated form of the particular residue within the site may be developed, and a second AQUA peptide incorporating the non-phosphorylated form of the residue developed. In this way, the two standards may be used to detect and quantify both the phosphorylated and non-phosphorylated forms of the site in a biological sample.

Peptide internal standards may also be generated by examining the primary amino acid sequence of a protein and determining the boundaries of peptides produced by protease cleavage. Alternatively, a protein may actually be digested with a protease and a particular peptide fragment produced can then sequenced. Suitable proteases include, but are not limited to, serine proteases (*e.g.* trypsin, hepsin), metallo proteases (*e.g.* PUMP1), chymotrypsin, cathepsin, pepsin, thermolysin, carboxypeptidases, etc.

A peptide sequence within a target protein is selected according to one or more criteria to optimize the use of the peptide as an internal

standard. Preferably, the size of the peptide is selected to minimize the chances that the peptide sequence will be repeated elsewhere in other non-target proteins. Thus, a peptide is preferably at least about 6 amino acids. The size of the peptide is also optimized to maximize ionization frequency. Thus, peptides longer than about 20 amino acids are not preferred. The preferred ranged is about 7 to 15 amino acids. A peptide sequence is also selected that is not likely to be chemically reactive during mass spectrometry, thus sequences comprising cysteine, tryptophan, or methionine are avoided.

A peptide sequence that does not include a modified region of the target region may be selected so that the peptide internal standard can be used to determine the quantity of all forms of the protein. Alternatively, a peptide internal standard encompassing a modified amino acid may be desirable to detect and quantify only the modified form of the target protein. Peptide standards for both modified and unmodified regions can be used together, to determine the extent of a modification in a particular sample (*i.e.* to determine what fraction of the total amount of protein is represented by the modified form). For example, peptide standards for both the phosphorylated and unphosphorylated form of a protein known to be phosphorylated at a particular site can be used to quantify the amount of phosphorylated form in a sample.

The peptide is labeled using one or more labeled amino acids (*i.e.* the label is an actual part of the peptide) or less preferably, labels may be attached after synthesis according to standard methods. Preferably, the label is a mass-altering label selected based on the following considerations: The mass should be unique to shift fragment masses produced by MS analysis to regions of the spectrum with low background; the ion mass signature component is the portion of the labeling moiety that preferably exhibits a unique ion mass signature in MS analysis; the sum of the masses of the constituent atoms of the label is preferably

uniquely different than the fragments of all the possible amino acids. As a result, the labeled amino acids and peptides are readily distinguished from unlabeled ones by the ion/mass pattern in the resulting mass spectrum. Preferably, the ion mass signature component imparts a mass to a protein fragment that does not match the residue mass for any of the 20 natural amino acids.

The label should be robust under the fragmentation conditions of MS and not undergo unfavorable fragmentation. Labeling chemistry should be efficient under a range of conditions, particularly denaturing conditions, and the labeled tag preferably remains soluble in the MS buffer system of choice. The label preferably does not suppress the ionization efficiency of the protein and is not chemically reactive. The label may contain a mixture of two or more isotopically distinct species to generate a unique mass spectrometric pattern at each labeled fragment position. Stable isotopes, such as ^{2}H, ^{13}C, ^{15}N, ^{17}O, ^{18}O, or ^{34}S, are among preferred labels. Pairs of peptide internal standards that incorporate a different isotope label may also be prepared. Preferred amino acid residues into which a heavy isotope label may be incorporated include leucine, proline, valine, and phenylalanine.

Peptide internal standards are characterized according to their mass-to-charge (m/z) ratio, and preferably, also according to their retention time on a chromatographic column (*e.g.* an HPLC column). Internal standards that co-elute with unlabeled peptides of identical sequence are selected as optimal internal standards. The internal standard is then analyzed by fragmenting the peptide by any suitable means, for example by collision-induced dissociation (CID) using, e.g., argon or helium as a collision gas. The fragments are then analyzed, for example by multi-stage mass spectrometry (MS^{n}) to obtain a fragment ion spectrum, to obtain a peptide fragmentation signature. Preferably, peptide fragments have significant differences in m/z ratios to enable

peaks corresponding to each fragment to be well separated, and a signature that is unique for the target peptide is obtained. If a suitable fragment signature is not obtained at the first stage, additional stages of MS are performed until a unique signature is obtained.

Fragment ions in the MS/MS and MS^3 spectra are typically highly specific for the peptide of interest, and, in conjunction with LC methods, allow a highly selective means of detecting and quantifying a target peptide/protein in a complex protein mixture, such as a cell lysate, containing many thousands or tens of thousands of proteins. Any biological sample potentially containing a target protein/peptide of interest may be assayed. Crude or partially purified cell extracts are preferably employed. Generally, the sample has at least 0.01 mg of protein, typically a concentration of 0.1-10 mg/mL, and may be adjusted to a desired buffer concentration and pH.

A known amount of a labeled peptide internal standard, preferably about 10 femtomoles, corresponding to a target protein to be detected/quantified is then added to a biological sample, such as a cell lysate. The spiked sample is then digested with one or more protease(s) for a suitable time period to allow digestion. A separation is then performed (*e.g.* by HPLC, reverse-phase HPLC, capillary electrophoresis, ion exchange chromatography, etc.) to isolate the labeled internal standard and its corresponding target peptide from other peptides in the sample. Microcapillary LC is a preferred method.

Each isolated peptide is then examined by monitoring of a selected reaction in the MS. This involves using the prior knowledge gained by the characterization of the peptide internal standard and then requiring the MS to continuously monitor a specific ion in the MS/MS or MS^n spectrum for both the peptide of interest and the internal standard. After elution, the area under the curve (AUC) for both peptide standard and target peptide peaks are calculated. The ratio of the two areas provides the absolute

quantification that can be normalized for the number of cells used in the analysis and the protein's molecular weight, to provide the precise number of copies of the protein per cell. Further details of the AQUA methodology are described in Gygi *et al.*, and Gerber *et al. supra*.

In accordance with the present invention, AQUA internal peptide standards (heavy-isotope labeled peptides) may now be produced, as described above, for either of the novel human ATR kinase protein phosphorylation sites disclosed herein. Peptide standards for a phosphorylation site (*e.g.* the serine 428 site in human ATR (see Figure 1)) may be produced for both the phosphorylated and non-phosphorylated forms of the site, and such standards employed in the AQUA methodology to detect and quantify both forms of such ATR phosphorylation site in a biological sample.

AQUA peptides of the invention may comprise an ATR peptide sequence, typically eight to forty amino acids in length, which encompasses the phosphorylatable serine (Ser428 or Ser2317) of interest (see Figure 1). For example, an AQUA peptide of the invention for detection/quantification of human ATR kinase when phosphorylated at serine 428 may consist of, or comprise, the sequence DGIs*PKRRRL (s*=phosphoserine), which comprises phosphorylatable serine 428 (*see* Figure 1) (SEQ ID NO: 1)). Longer peptides corresponding to particular digestion fragments may be desirable, and exemplary fragments are described in Example 4 below. Heavy-isotope labeled equivalents of a particular ATR peptide (encompassing either the Ser428 or Ser2317 sites disclosed herein) can be readily synthesized, both in phosphorylated and unphosphorylated form, and their unique MS and LC-SRM signature determined, so that the peptides are validated as AQUA peptides and ready for use in quantification experiments. AQUA peptides for quantifying or detecting highly homologous ATR phospho-sites in other species (*e.g.* mouse, as disclosed herein) may similarly be constructed.

Particularly preferred ATR peptides suitable for development of corresponding AQUA peptides are those peptides (encompassing either serine 428 or serine 2317) that are produced by enzymatic digestion (*e.g.* with trypsin, GluC, AspN, etc.) of ATR (SEQ ID NO: 1). Heavy-isotope labeled equivalents of these peptides (both in phosphorylated and unphosphorylated form) can be readily synthesized and their unique MS and LC-SRM signature determined, so that the peptides are validated as AQUA peptides and ready for use in quantification experiments. Such digestion fragments may be shorter or larger ATR peptides, and the selection and production of preferred AQUA peptides may be carried out as described above (*see* Gygi *et al.*, Gerber *et al. supra.*). Two exemplary digestion fragments are described in Example 4 below.

AQUA peptides of the invention may also be employed within a kit that comprises one or multiple AQUA peptide(s) provided, and, optionally, a second detecting reagent conjugated to a detectable group. For example, a kit may include AQUA peptides for both the phosphorylated and non-phosphorylated form of a phosphorylation site disclosed herein. The reagents may also include ancillary agents such as buffering agents and protein stabilizing agents, *e.g.*, polysaccharides and the like. The kit may further include, where necessary, enzyme substrates, agents for reducing background interference in a test, control reagents, apparatus for conducting a test, and the like. The test kit may be packaged in any suitable manner, typically with all elements in a single container along with a sheet of printed instructions for carrying out the test.

AQUA peptides provided by the invention will be highly useful, among other things, in the further study of ATR kinase signaling, aberrations in ATR activity underlying disease, and/or in monitoring the effects of test compounds on ATR and ATR-mediated signal transduction proteins and pathways.

D. Detection & Profiling Methods

The methods disclosed herein may be employed with any biological sample potentially containing, or suspected of containing, phosphorylated ATR kinase. Biological samples taken from a mammal, *e.g.* a human a subject, for use in the methods disclosed herein are generally biological fluids such as serum, blood plasma, fine needle aspirant, ductal lavage, bone marrow sample or ascites fluid. In the alternative, the sample taken from the subject can be a tissue sample (*e.g.*, a biopsy tissue), such as bone marrow or tumor tissue, or a cell lysate, whether or not purified.

In one embodiment, the invention provides a method for detecting phosphorylated ATR in a biological sample by (a) contacting a biological sample potentially (or suspected of) containing phosphorylated ATR and/or phosphorylated ATR with at least one detectable reagent that binds to and/or detects human ATR only when phosphorylated at Ser428 or Ser2317, respectively, under conditions suitable for formation of a reagent-ATR complex, and (b) detecting the presence of the complex in the sample, wherein the presence of the complex indicates the presence of phosphorylated ATR (Ser428) and/or phosphorylated ATR (Ser2317) in the sample.

In a preferred embodiment, the reagent is an ATR phospho-specific antibody of the invention. In another preferred embodiment, the reagent is an ATR heavy isotope-labeled peptide (AQUA peptide of the invention. In other preferred embodiments, the biological sample has been contacted with at least one modulator of ATR activity, *e.g.* an ATR inhibitor, or is obtained from a subject treated with such modulator. Changes in ATR(Ser428 and/or Ser2317) phosphorylation resulting from contacting a biological sample with a test compound, such as an AKT inhibitor, may be examined to determine the effect of such compound. The compound may be a "pan" kinase inhibitor that inhibits more than

type of kinase including ATR (for example, bis-indoleimide), or may be a specific inhibitor of ATR kinase. The inhibitor may inhibit the expression and/or activity of ATR. Exemplary inhibitors of ATR include, but are not limited to, caffeine.

Inhibitory compounds may be targeted inhibitors that modulate post-translational activity of ATR, or may be upstream expression inhibitors, such as siRNA or anti-sense inhibitors. In another preferred embodiment, the compound is being tested for inhibition of ATR activity or expression. Such compound may, for example, directly inhibit ATR activity, or may indirectly inhibit its activity by, *e.g.*, inhibiting another kinase that phosphorylates and thus activates ATR, or by inhibiting co-factors or necessary binding partners or complex partners of ATR. Likewise, modulators that enhance the activity and/or expression of ATR may be similarly targeted or general modulators, or direct or indirect modulators, as described above.

Biological samples may be obtained from subjects at risk of, potentially, or suspected of, having a disease or condition involving altered ATR expression or activity (*e.g.*, Seckel syndrome). For example, samples may be analyzed to monitor subjects who have been previously diagnosed as having Seckel syndrome, to screen subjects who have not been previously diagnosed as having this disease, or to monitor the desirability or efficacy of therapeutics targeted at modulating the activity of ATR.

In another embodiment, the invention provides a method for profiling ATR phosphorylation in a test tissue potentially having (or suspected of involving) altered ATR expression and/or activity, by (a) contacting the test tissue with at least one detectable reagent that binds to and/or detects human ATR only when phosphorylated at Ser428 or Ser2317, respectively, under conditions suitable for formation of a reagent-ATR complex, (b) detecting the presence of the complex in the

test tissue, wherein the presence of the complex indicates the presence of phosphorylated ATR (Ser428) or phosphorylated ATR (Ser2317) in the test tissue, and (c) comparing the presence of phosphorylated ATR detected in step(b) with the presence of phosphorylated ATR (Ser428, Ser2317) in a control tissue, wherein a difference in ATR phosphorylation profiles between the test and control tissues indicates altered ATR expression and/or activation in the test tissue. In a preferred embodiment, the reagent is an ATR phospho-specific antibody of the invention. In another preferred embodiment, the reagent is an ATR AQUA peptide of the invention.

The methods described above are applicable to examining tissues or samples from any disease or condition involving or characterized by altered ATR expression and/or activity, in which phosphorylation of ATR at Ser428 and/or Ser2317, respectively, (and possibly other serine residues) has predictive value as to the outcome of the disease or the response of the disease to therapy. It is anticipated that the ATR antibodies and AQUA peptides will have diagnostic utility in a disease characterized by, or involving, altered ATR phosphorylation and/or signaling. The methods are applicable, for example, where samples are taken from a subject has not been previously diagnosed as having a disease characterized by altered ATR expression and/or activity, nor has yet undergone treatment for the disease, and the method is employed to help diagnose the disease, or monitor the possible progression of the condition, or assess risk of the subject developing disease involving altered ATR (Ser428, Ser2317) phosphorylation.

Such diagnostic assay may be carried out prior to preliminary blood, fluid, or tissue evaluation or surgical surveillance procedures. Such a diagnostic assay may be employed to identify patients with activated or inhibited ATR, who would be most likely to respond to therapeutics targeted at activating or inhibiting ATR activity. Such a

selection of patients would be useful in the clinical evaluation of efficacy of future ATR–targeted therapeutics as well as in the future prescription of such drugs to patients. Alternatively, the methods are applicable where a subject has been previously diagnosed as having a disease involving altered ATR signaling, such as Seckel syndrome, and possibly has already undergone treatment for the disease, and the method is employed to monitor the progression of the disease, or the treatment thereof.

In another embodiment, the invention provides a method for identifying a compound which modulates phosphorylation of ATR in a test tissue, by (a) contacting the test tissue with the compound, (b) detecting the level of phosphorylated ATR and/or ATR in said the test tissue of step (a) using at least one detectable reagent that binds to and/or detects ATR when phosphorylated at Ser428 or Ser2317 under conditions suitable for formation of a reagent-ATR complex, and (c) comparing the level of phosphorylated ATR and/or ATR detected in step(b) with the presence of phosphorylated ATR (Ser428, Ser2317) in a control tissue not contacted with the compound, wherein a difference in ATR phosphorylation levels between the test and control tissues identifies the compound as a modulator of ATR phosphorylation. In a preferred embodiment, the reagent is an ATR antibody or an AQUA peptide of the invention. The compound may modulate ATR activity either positively or negatively, for example by increasing or decreasing phosphorylation or expression of ATR. Alternatively, ATR phosphorylation may be monitored to determine the efficacy of a compound targeted at any kinase that phosphorylates ATR (Ser428) and/or ATR (Ser2317), or any phosphatase that de-phosphorylates ATR at one or both of these sites.

Conditions suitable for the formation of antibody-antigen complexes or reagent-ATR complexes are well known in the art (*see* part (d) below and references cited therein). It will be understood that more

than one ATR phospho-specific antibody may be used in the practice of the above-described methods. For example, a phospho-ATR (Ser428, Ser2317) antibody and a phospho-specific antibody to another serine, tyrosine, or threonine phosphorylation site may be simultaneously employed to detect phosphorylation of both sites in one step.

E. Immunoassay Formats & Diagnostic Kits

Assays carried out in accordance with methods of the present invention may be homogeneous assays or heterogeneous assays. In a homogeneous assay the immunological reaction usually involves an ATR-specific reagent (*e.g.* a ATR phospho-antibody of the invention), a labeled analyte, and the sample of interest. The signal arising from the label is modified, directly or indirectly, upon the binding of the antibody to the labeled analyte. Both the immunological reaction and detection of the extent thereof are carried out in a homogeneous solution. Immuno-chemical labels that may be employed include free radicals, radio-isotopes, fluorescent dyes, enzymes, bacteriophages, coenzymes, and so forth.

In a heterogeneous assay approach, the reagents are usually the specimen, an ATR-specific reagent (*e.g.*, the ATR antibody of the invention), and suitable means for producing a detectable signal. Similar specimens as described above may be used. The antibody is generally immobilized on a support, such as a bead, plate or slide, and contacted with the specimen suspected of containing the antigen in a liquid phase. The support is then separated from the liquid phase and either the support phase or the liquid phase is examined for a detectable signal employing means for producing such signal. The signal is related to the presence of the analyte in the specimen. Means for producing a detectable signal include the use of radioactive labels, fluorescent labels, enzyme labels, and so forth. For example, if the antigen to be detected

contains a second binding site, an antibody which binds to that site can be conjugated to a detectable group and added to the liquid phase reaction solution before the separation step. The presence of the detectable group on the solid support indicates the presence of the antigen in the test sample. Examples of suitable immunoassays are the radioimmunoassay, immunofluorescence methods, enzyme-linked immunoassays, and the like.

Immunoassay formats and variations thereof, which may be useful for carrying out the methods disclosed herein, are well known in the art. *See generally* E. Maggio, Enzyme-Immunoassay, (1980) (CRC Press, Inc., Boca Raton, Fla.); *see also, e.g.,* U.S. Pat. No. 4,727,022 (Skold *et al.,* "Methods for Modulating Ligand-Receptor Interactions and their Application"); U.S. Pat. No. 4,659,678 (Forrest *et al.,* "Immunoassay of Antigens"); U.S. Pat. No. 4,376,110 (David *et al.*, "Immunometric Assays Using Monoclonal Antibodies"). Conditions suitable for the formation of reagent-antibody complexes are well described. *See id.* Monoclonal antibodies of the invention may be used in a "two-site" or "sandwich" assay, with a single cell line serving as a source for both the labeled monoclonal antibody and the bound monoclonal antibody. Such assays are described in U.S. Pat. No. 4,376,110. The concentration of detectable reagent should be sufficient such that the binding of phosphorylated ATR is detectable compared to background.

ATR antibodies disclosed herein may be conjugated to a solid support suitable for a diagnostic assay (*e.g.*, beads, plates, slides or wells formed from materials such as latex or polystyrene) in accordance with known techniques, such as precipitation. Antibodies of the invention, or other ATR binding reagents, may likewise be conjugated to detectable groups such as radiolabels (*e.g.*, ^{35}S, ^{125}I, ^{131}I), enzyme labels (*e.g.*, horseradish peroxidase, alkaline phosphatase), and fluorescent labels (*e.g.*, fluorescein) in accordance with known techniques.

ATR phospho-specific antibodies of the invention may also be used in a flow cytometry assay to determine the activation status of ATR in patients before, during, and after treatment with a drug targeted at inhibiting ATR phosphorylation at Ser428 and/or Ser2317. For example, bone marrow cells or peripheral blood cells from patients may be analyzed by flow cytometry for ATR phosphorylation, as well as for markers identifying various hematopoietic cell types. In this manner, ATR activation status of the diseased cells may be specifically characterized.

Flow cytometry may be carried out according to standard methods. *See, e.g.* Chow *et al.*, *Cytometry (Communications in Clinical Cytometry) 46:* 72–78 (2001). Briefly and by way of example, the following protocol for cytometric analysis may be employed: fixation of the cells with 2% paraformaldehyde for 20 minutes at 37 °C followed by permeabilization in 90% methanol for 30 minutes on ice. Cells may then be stained with the primary ATR antibody, washed and labeled with a fluorescent-labeled secondary antibody. The cells would then be analyzed on a flow cytometer (*e.g.* a Beckman Coulter EPICS-XL) according to the specific protocols of the instrument used. Such an analysis would identify the presence of phosphorylated ATR (Ser428, Ser2317) in a cell of interest and reveal the drug response on the targeted kinase.

Diagnostic kits for carrying out the methods disclosed above are also provided by the invention. Such kits comprise at least one detectable reagent that binds to or detects human ATR only when phosphorylated at Ser428 or Ser2317, respectively. In a preferred embodiment, the reagent is an ATR phospho-specific antibody of the invention. In another preferred embodiment, the reagent is an ATR AQUA peptide of the invention. In one embodiment, the invention provides a kit for the detection of phosphorylated ATR (Ser428) and/or ATR (Ser2317) in a biological sample comprising at least one ATR specific reagent of the invention (*i.e.* a phospho-specific antibody that

binds phospho-ATR (Ser428, Ser2317)). The kit may also include one or more secondary reagents, such as a secondary antibody, or ancillary agents such as buffering agents and protein stabilizing agents, *e.g.*, polysaccharides and the like. The diagnostic kit may further include, where necessary, other enzyme substrates, agents for reducing background interference in a test, control reagents, apparatus for conducting a test, and the like.

The following Examples are provided only to further illustrate the invention, and are not intended to limit its scope, except as provided in the claims appended hereto. The present invention encompasses modifications and variations of the methods taught herein which would be obvious to one of ordinary skill in the art.

EXAMPLE 1

Production of a Human ATR (Ser428) Phospho-Specific Antibody

A previously unknown ATR phosphorylation site, serine 428, was identified as described above by predictive analysis of the human ATR protein sequence using the ScanSite program. Yaffe *et al.*, *supra.* A 13 amino acid phospho-peptide antigen, SNSDGI***S**PKRRRL (SEQ ID NO: 5) (where ***S** = phosphoserine), corresponding to residues 422-434 of human ATR (*see* SEQ ID NO: 1; Figure 1), was constructed according to standard synthesis techniques using a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer. *See* ANTIBODIES: A LABORATORY MANUAL, *supra.;* Merrifield, *supra.* This peptide antigen also corresponds to a highly homologous site in murine ATR (*see* Figure 2; SEQ ID NO: 2).

This peptide was coupled to KLH, and rabbits were injected intradermally (ID) on back with antigen in complete Freunds adjuvant (500 μg antigen per rabbit). The rabbits were boosted with same antigen in incomplete Freund adjuvant (250 μg antigen per rabbit) every three

weeks. After the fifth boost, the bleeds were collected. The sera were purified by Protein A-sepharose affinity chromatography as previously described (*see* ANTIBODIES: A LABORATORY MANUAL, Cold Spring Harbor, *supra.*). Further purification steps were performed using adsorption of specific material to phosphopeptide and nonphosphopeptide affinity columns, followed by elution of reactive material at low pH, as follows. The protein A-purified immunoglobulins were loaded onto a SNSDGI*SPKRRRL–resin column. The bound antibodies were eluted at low pH, collected, and applied onto a SNSDGISPKRRRL–resin column. The flow through fraction was collected, dialyzed, and kept in storage buffer.

Antibodies were characterized by Western blotting to examine specificity against whole cell extracts, as described in detail below.

Characterization of p-ATR (Ser428) Antibodies Against Phosphorylated ATR in DNA Damaged Cell Lines and Confirmation of *In Vivo* Phosphorylation of Ser428.

To characterize the polyclonal antibodies raised against the phosphorylated peptide described above, Western blots were performed with RAW 264.7 cells, which were known to give a strong signal transduction response (ATR-p53 pathway) to DNA damage (*see Free Radic. Biol. Med. 30*: 884-94 and *Biochem. J. 319:* 299-305). The cells were either untreated (control) or treated with either UV (50 mJ/cm^2, 30 minute recovery) in order to induce DNA damage and stimulate ATR activity, or Nocadazole (50 ng/mL, 16 hours) in order to arrest cells in metaphase of mitosis. Total ATR was detecting using a total ATR-specific antibody (Novus Biologicals cat# NB 100-322).

Figure 3 shows the specific detection of human ATR (phosphorylated at Ser428) by the ATR(Ser428) phospho-specific antibody of the invention. Basal phospho-ATR in untreated cells is detected (lane 1), and increase in phosphorylated ATR is detected

following UV treatment (lane 2), but not following Nocadazole treatment (lane 3). Analysis of total ATR protein served as a control to indicate equal loading and to verify that the stimuli applied worked as expected. These results confirm that the novel serine 428 ATR phosphorylation site disclosed herein is, in fact, phosphorylated *in vivo*.

Characterization of p-ATR (Ser428) Antibodies Using ATR Substitution Mutants.

To further examine the specificity of phospho-ATR(Ser428) antibodies, expression constructs encoding epitope (HA) tagged wild-type ATR protein, or ATR containing an amino acid substitution at position 428 (for example serine-to-alanine) in the human sequence may be prepared by transfecting NIH 3T3 cells, according to standard methods (*See* Qiagen Polyfect® Transfection Reagent Handbook, Sept. 2000).

Transfected cells may be UV treated to induce DNA damage, and hence ATR activation, and HA-ATR proteins may be immunoprecipitated using an anti-HA antibody. The immunoprecipitated material is then immunoblotted using a phospho-ATR(Ser428) antibody. This analysis is useful to further confirm that a phospho-ATR (Ser428) antibody will detect the wild-type ATR protein, but not the mutant ATR having the Ser428 substitution. Anti-HA antibodies are used to control for the amounts of total ATR protein immunoprecipitated and immunoblotted in this type of experiment, and a total ATR immunoblot is used to indicate that the UV treatment worked as expected leading to activation, and hence phosphorylation, of ATR.

EXAMPLE 2

Production of a Human ATR (Ser2317) Phospho-Specific Antibody

A previously unknown ATR phosphorylation site, serine 2317, was identified as described above by predictive analysis of the human ATR protein sequence using the ScanSite program. Yaffe *et al.*, *supra.* A 13 amino acid phospho-peptide antigen, ISLKG***S**DGKFY (SEQ ID NO: 6) (where ***S** = phosphoserine), corresponding to residues 2312-2322 of human ATR (*see* SEQ ID NO: 1; Figure 1), was constructed according to standard synthesis techniques using a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer. *See* ANTIBODIES: A LABORATORY MANUAL, *supra.;* Merrifield, *supra.* This peptide antigen also corresponds to a highly homologous site in murine ATR (*see* Figure 2; SEQ ID NO: 2).

This peptide was coupled to KLH, and rabbits were injected intradermally (ID) on back with antigen in complete Freunds adjuvant (500 μg antigen per rabbit). The rabbits were boosted with same antigen in incomplete Freund adjuvant (250 μg antigen per rabbit) every three weeks. After the fifth boost, the bleeds were collected. The sera were purified by Protein A-sepharose affinity chromatography as previously described (*see* ANTIBODIES: A LABORATORY MANUAL, Cold Spring Harbor, *supra.*). Further purification steps were performed using adsorption of specific material to phosphopeptide and nonphosphopeptide affinity columns, followed by elution of reactive material at low pH, as follows. The protein A-purified immunoglobulins were loaded onto a ISLKG***S**DGKFY–resin Knotes column. The bound antibodies were eluted at low pH, collected, and applied onto a ISLKGSDGKFY–resin column. The flow through fraction was collected, dialyzed, and kept in storage buffer.

Antibodies were characterized by Western blotting to examine specificity against whole cell extracts, as described in detail below.

Characterization of p-ATR (Ser2317) Antibodies Against Phosphorylated ATR in DNA Damaged Cell Lines and Confirmation of In Vivo Phosphorylation of Ser2317.

To characterize the polyclonal antibodies raised against the phosphorylated peptide described above, Western blots were performed with 264.7 cells, which were known to give a strong signal transduction response (ATR-p53 pathway) to DNA damage (*see Free Radic. Biol. Med. 30:, supra.*) The cells were either untreated (control) or treated with either UV (50 mJ/cm^2, 30 minute recovery) in order to induce DNA damage and stimulate ATR activity, or Nocadazole (50 ng/mL, 16 hours) in order to arrest cells in metaphase of mitosis. Total ATR was detecting using a total ATR-specific antibody (Novus Biologicals cat# NB 100-322).

Figure 3 shows the specific detection of human ATR (phosphorylated at Ser2317) by the ATR(Ser2317) phospho-specific antibody of the invention. Basal phospho-ATR is untreated cells is detected (lane 1), and increase in phosphorylated ATR is detected following nocodazole-block (lane 3), but not following UV-treatment (lane 2). Analysis of total ATR protein served as a control to indicate equal loading and to verify that the stimuli applied worked as expected. These results confirm that the novel serine 2317 ATR phosphorylation site disclosed herein is, in fact, phosphorylated *in vivo*.

Characterization of p-ATR (Ser2317) Antibodies Using ATR Substitution Mutants.

To further examine the specificity of phospho-ATR(Ser2317) antibodies, expression constructs encoding epitope (HA) tagged wild-type ATR protein, or ATR containing an amino acid substitution at position 2317 (for example serine-to-alanine) in the human sequence

may be prepared by transfecting NIH 3T3 cells, according to standard methods (*See* Qiagen Polyfect® Transfection Reagent Handbook, Sept. 2000).

Transfected cells may be UV treated to induce DNA damage, and hence ATR activation, and HA-ATR proteins may be immunoprecipitated using an anti-HA antibody. The immunoprecipitated material is then immunoblotted using a phospho-ATR(Ser2317) antibody. This analysis is useful to further confirm that a phospho-ATR (Ser2317) antibody will detect the wild-type ATR protein, but not the mutant ATR having the Ser2317 substitution. Anti-HA antibodies are used to control for the amounts of total ATR protein immunoprecipitated and immunoblotted in this type of experiment, and a total ATR immunoblot is used to indicate that the UV treatment worked as expected leading to activation, and hence phosphorylation, of ATR.

EXAMPLE 3

Production of a Human ATR (Ser428, Ser2317) Phospho-Specific Monoclonal Antibody

Phospho-ATR (Ser428 or Ser2317)–specific monoclonal antibodies may be produced from spleen cells of the immunized BALB/c mice described in Examples 1 and 2, above, following standard procedures (Harlow and Lane, 1988). Briefly, the mouse spleen is fused to SP2/0 mouse myeloma fusion partner cells according to the protocol of Kohler and Milstein (1975). Colonies originating from the fusion are screened by ELISA for reactivity to the phospho-peptide and non-phospho-peptide and by Western blot analysis. Colonies found to be positive by ELISA to the phospho-peptide while negative to the non-phospho-peptide may be further characterized by Western blot analysis. Colonies found to be positive by Western blot analysis are then subcloned by limited dilution. Mouse ascites are produced from positive clones

obtained from subcloning. Clones are selected for phospho-specificity by ELISA and by Western blot analysis using cell culture supernatant. Selected positive clones are then subcloned to produce final desired clones producing phospho-ATR (Ser428, Ser2317)-specific monoclonal antibodies.

Ascites fluid from clones obtained from the ATR fusion may be further tested by Western blot analysis. The ascites fluid will likely give similar results on Western blot analysis as observed with the cell culture supernatant, indicating phospho-specificity on ATR-induced 3T3L1 adipocytes and/or L6 differentiated myocyte cells, for example.

EXAMPLE 4

Production and Use of AQUA Peptides for the Quantification of ATR (Ser428, Ser2317) Protein Phosphorylation

Heavy-isotope labeled peptides (AQUA peptides (internal standards)) for the detection and quantification of ATR kinase only when phosphorylated at Ser428 and/or Ser2317 are produced according to the standard AQUA methodology (*see* Gygi *et al.*, Gerber *et al., supra.*) methods by first constructing a synthetic peptide standard corresponding to the respective phosphorylation site sequence (*see* Figure 1, underlines) and incorporating a heavy-isotope label. Subsequently, the MS^n and LC-SRM signature of the peptide standard is validated, and the AQUA peptide is used to quantify native peptide in a biological sample, such as a digested cell extract. Production and use of exemplary AQUA peptides is provided below.

ATR (serine 428).

An AQUA peptide comprising the sequence, NLSSNSDGIs*PKRRRLSSSLNPSKRA**P**KQTE (s*= phosphoserine; sequence incorporating $^{14}C/^{15}N$-labeled proline (indicated by bold **P**)

(SEQ ID NO: 7), which corresponds to the serine 428 phosphorylation site in human ATR kinase (*see* Figure 1 (SEQ ID NO: 1), residues 419-449), is constructed according to standard synthesis techniques using, *e.g.*, a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer (*see* Merrifield, *supra.*) as further described below in Synthesis & MS/MS Signature. This ATR peptide corresponds to a predicted GluC digest fragment. The ATR (Ser428) AQUA peptide is then spiked into a biological sample to quantify the amount of phosphorylated ATR(Ser428) in the sample, as further described below in Analysis & Quantification.

ATR (serine 2317).

An AQUA peptide comprising the sequence DMVEILASLQKPKKISLKGs*DGKFYIMMCK**P**K (s*= phosphoserine; sequence incorporating $^{14}C/^{15}N$-labeled proline (indicated by bold **P**) (SEQ ID NO: 8), which corresponds to the serine 2317 phosphorylation site in human ATR kinase (*see* Figure 1 (SEQ ID NO: 1), residues 2298-2329), is constructed according to standard synthesis techniques using, *e.g.*, a Rainin/Protein Technologies, Inc., Symphony peptide synthesizer (*see* Merrifield, *supra.*) as further described below in Synthesis & MS/MS Signature. This ATR peptide corresponds to a predicted AspN digestion fragment. The ATR(Ser2317) AQUA peptide is then spiked into a biological sample to quantify the amount of phosphorylated ATR (Ser2317) in the sample, as further described below in Analysis & Quantification.

Synthesis & MS/MS Spectra.

Fluorenylmethoxycarbonyl (Fmoc)-derivatized amino acid monomers may be obtained from AnaSpec (San Jose, CA). Fmoc-derivatized stable-isotope monomers containing one ^{15}N and five to nine ^{13}C atoms may be obtained from Cambridge Isotope Laboratories (Andover, MA). Preloaded

Wang resins may be obtained from Applied Biosystems. Synthesis scales may vary from 5 to 25 µmol. Amino acids are activated *in situ* with 1-H-benzotriazolium, 1-bis(dimethylamino) methylene]-hexafluorophosphate (1-),3-oxide:1-hydroxybenzotriazole hydrate and coupled at a 5-fold molar excess over peptide. Each coupling cycle is followed by capping with acetic anhydride to avoid accumulation of one-residue deletion peptide by-products. After synthesis peptide-resins are treated with a standard scavenger-containing trifluoroacetic acid (TFA)-water cleavage solution, and the peptides are precipitated by addition to cold ether. Peptides (*i.e.* a desired ATR AQUA peptide described above) are purified by reversed-phase C18 HPLC using standard TFA/acetonitrile gradients and characterized by matrix-assisted laser desorption ionization-time of flight (Biflex III, Bruker Daltonics, Billerica, MA) and ion-trap (ThermoFinnigan, LCQ DecaXP) MS.

MS/MS spectra for each AQUA peptide should exhibit a strong *y*-type ion peak as the most intense fragment ion that is suitable for use in an SRM monitoring/analysis. Reverse-phase microcapillary columns (0.1 Å~ 150–220 mm) are prepared according to standard methods. An Agilent 1100 liquid chromatograph may be used to develop and deliver a solvent gradient [0.4% acetic acid/0.005% heptafluorobutyric acid (HFBA)/7% methanol and 0.4% acetic acid/0.005% HFBA/65% methanol/35% acetonitrile] to the microcapillary column by means of a flow splitter. Samples are then directly loaded onto the microcapillary column by using a FAMOS inert capillary autosampler (LC Packings, San Francisco) after the flow split. Peptides are reconstituted in 6% acetic acid/0.01% TFA before injection.

Analysis & Quantification.

Target protein (phosphorylated ATR kinase) in a biological sample is quantified using a validated AQUA peptide (as described above). The

IAP method is then applied to the complex mixture of peptides derived from proteolytic cleavage of crude cell extracts to which the AQUA peptides have been spiked in.

LC–SRM of the entire sample is then carried out. MS/MS may be performed by using a ThermoFinnigan (San Jose, CA) mass spectrometer (LCQ DecaXP ion trap or TSQ Quantum triple quadrupole). On the DecaXP, parent ions are isolated at 1.6 m/z width, the ion injection time being limited to 150 ms per microscan, with two microscans per peptide averaged, and with an AGC setting of 1×10^8; on the Quantum, Q1 is kept at 0.4 and Q3 at 0.8 m/z with a scan time of 200 ms per peptide. On both instruments, analyte and internal standard are analyzed in alternation within a previously known reverse-phase retention window; well-resolved pairs of internal standard and analyte are analyzed in separate retention segments to improve duty cycle. Data are processed by integrating the appropriate peaks in an extracted ion chromatogram (60.15 m/z from the fragment monitored) for the native and internal standard, followed by calculation of the ratio of peak areas multiplied by the absolute amount of internal standard (*e.g.*, 500 fmol).

WHAT IS CLAIMED IS:

1. An isolated antibody that binds to human Ataxia-Telangiectasia and Rad3-related (ATR) kinase when phosphorylated at serine 428 (SEQ ID NO: 1) or when phosphorylated at serine 2317 (SEQ ID NO: 1), but does not bind human ATR when not phosphorylated at either of these respective positions.

2. The antibody of claim 1, wherein said antibody further binds to murine ATR when phosphorylated at serine 419 (SEQ ID NO: 2) and/or murine ATR when phosphorylated at serine 2296 (SEQ ID NO: 2), but does not bind murine ATR when not phosphorylated at either of these respective positions.

3. The antibody of claim 1, wherein said antibody is polyclonal.

4. The antibody of claim 1, wherein said antibody is monoclonal.

5. An immortalized cell line producing the antibody of claim 4.

6. A heavy-isotope labeled peptide (AQUA peptide) for the quantification of human ATR kinase, said labeled peptide comprising an ATR peptide encompassing either the serine 428 or serine 2317 phosphorylation site sequences (SEQ ID NO: 1).

7. The labeled peptide of claim 6, wherein the serine 428 or serine 2317 residue comprised within said peptide is phosphorylated.

8. The labeled peptide of claim 6, wherein said peptide consists of the sequence of SEQ ID NO: 7 or SEQ ID NO: 8.

9. The labeled peptide of claim 6, wherein the serine 428 or serine 2317 residue comprised within said peptide is not phosphorylated.

10. A method for detecting phosphorylated ATR kinase in a biological sample, said method comprising the steps of:

(a) contacting a biological sample potentially, or suspected of, containing phosphorylated ATR kinase with at least one detectable ATR(Ser428, Ser2317)-specific reagent, under conditions suitable for formation of a reagent-ATR complex; and

(b) detecting the presence of said complex in said sample, wherein the presence of said complex indicates the presence of phosphorylated ATR (Ser428, Ser2317) in said sample.

11. The method of claim 10, wherein said detectable ATR (Ser428, Ser2317)-specific reagent comprises the isolated antibody of claim 1.

12. The method of claim 10, wherein said detectable ATR (Ser428, Ser2317)-specific reagent comprises the heavy isotope-labeled peptide of claim 6.

13. The method of claim 10, wherein said biological sample has been contacted with at least one ATR modulator, or is obtained from a subject treated with such modulator.

14. The method of claim 10, wherein said biological sample has been contacted with a compound being tested for modulation of ATR activity or expression.

15. A kit for the detection of phosphorylated ATR (Ser428) and/or phosphorylated ATR (Ser2317) in a biological sample, said kit comprising at least one detectable antibody of claim 1 and/or at least one heavy isotope-labeled peptide of claim 6.

Figure 1.

MGEHGLELASMIPALRELGSATPEEYNTVVQKPRQILCQFIDRILTDVNVVAVELVKKTD
SQPTSVMLLDFIQHIMKSSPLMFVNVSGSHEAKGSCIEFSNWIITRLLRIAATPSCHLLH
KKICEVICSLLFLFKSKSPAIFGVLTKELLQLFEDLVYLHRRNVMGHAVEWPVVMSRFLS
QLDEHMGYLQSAPLQLMSMQNLEFIEVTLLMVLTRIIAIVFFRRQELLLWQIGCVLLEYG
SPKIKSLAISFLTELFQLGGLPAQPASTFFSSFLELLKHLVEMDTDQLKLYEEPLSKLIK
TLFPFEAEAYRNIEPVYLNMLLEKLCVMFEDGVLMRLKSDLLKAALCHLLQYFLKFVPAG
YESALQVRKVYVRNICKALLDVLGIEVDAEYLLGPLYAALKMESMEIIEEIQCQTQQENL
SSNSDGISPKRRRLSSSLNPSKRAPKQTEEIKHVDMNQKSILWSALKQKAESLQISLEYS
GLKNPVIEMLEGIAVVLQLTALCTVHCSHQNMNCRTFKDCQHKSKKKPSVVITWMSLDFY
TKVLKSCRSLLESVQKLDLEATIDKVVKIYDALIYMQVNSSFEDHILEDLCGMLSLPWIY
SHSDDGCLKLTTFAANLLTLSCRISDSYSPQAQSRCVFLLTLFPRRIFLEWRTAVYNWAL
QSSHEVIRASCVSGFFILLQQQNSCNRVPKILIDKVKDDSDIVKKEFASILGQLVCTLHG
MFYLTSSLTEPFSEHGHVDLFCRNLKATSQHECSSSQLKASVCKPFLFLLKKKIPSPVKL
AFIDNLHHLCKHLDFREDETDVKAVLGTLLNLMEDPDKDVRVAFSGNIKHILESLDSEDG
FIKELFVLRMKEAYTHAQISRNNELKDTLILTTGDIGRAAKGDLVPFALLHLLHCLLSKS
ASVSGAAYTEIRALVAAKSVKLQSFFSQYKKPICQFLVESLHSSQMTALPNTPCQNADVR
KQDVAHQREMALNTLSEIANVFDFPDLNRFLTRTLQVLLPDLAAKASPAASALIRTLGKQ
LNVNRREILINNFKYIFSHLVCSCSKDELERALHYLKNETEIELGSLLRQDFQGLHNELL
LRIGEHYQQVFNGLSILASFASSDDPYQGPRDIISPELMADYLQPKLLGILAFFNMQLLS
SSVGIEDKKMALNSLMSLMKLMGPKHVSSVRVKMMTTLRTGLRFKDDFPELCCRAWDCFV
RCLDHACLGSLLSHVIVALLPLIHIQPKETAAIFHYLIIENRDAVQDFLHEIYFLPDHPE
LKKIKAVLQEYRKETSESTDLQTTLQLSMKAIQHENVDVRIHALTSLKETLYKNQEKLIK
YATDSETVEPIISQLVTVLLKGCQDANSQARLLCGECLGELGAIDPGRLDFSTTETQGKD
FTFVTGVEDSSFAYGLLMELTRAYLAYADNSRAQDSAAYAIQELLSIYDCREMETNGPGH
QLWRRFPEHVREILEPHLNTRYKSSQKSTDWSGVKKPIYLSKLGSNFAEWSASWAGYLIT
KVRHDLASKIFTCCSIMMKHDFKVTIYLLPHILVYVLLGCNQEDQQEVYAEIMAVLKHDD
QHTINTQDIASDLCQLSTQTVFSMLDHLTQWARHKFQALKAEKCPHSKSNRNKVDSMVST
VDYEDYQSVTRFLDLIPQDTLAVASFRSKAYTRAVMHFESFITEKKQNIQEHLGFLQKLY
AAMHEPDGVAGVSAIRKAEPSLKEQILEHESLGLLRDATACYDRAIQLEPDQIIHYHGVV
KSMLGLGQLSTVITQVNGVHANRSEWTDELNTYRVEAAWKLSQWDLVENYLAADGKSTTW
SVRLGQLLLSAKKRDITAFYDSLKLVRAEQIVPLSAASFERGSYQRGYEYIVRLHMLCEL
EHSIKPLFQHSPGDSSQEDSLNWVARLEMTQNSYRAKEPILALRRALLSLNKRPDYNEMV
GECWLQSARVARKAGHHQTAYNALLNAGESRLAELYVERAKWLWSKGDVHQALIVLQKGV
ELCFPENETPPEGKNMLIHGRAMLLVGRFMEETANFESNAIMKKYKDVTACLPEWEDGHF
YLAKYYDKLMPMVTDNKMEKQGDLIRYIVLHFGRSLQYGNQFIYQSMPRMLTLWLDYGTK
AYEWEKAGRSDRVQMRNDLGKINKVITEHTNYLAPYQFLTAFSQLISRICHSHDEVFVVL
MEIIAKVFLAYPQQAMWMMTAVSKSSYPMRVNRCKEILNKAIHMKKSLEKFVGDATRLTD
KLLELCNKPVDGSSSTLSMSTHFKMLKKLVEEATFSEILIPLQSVMIPTLPSILGTHANH
ASHEPFPGHWAYIAGFDDMVEILASLQKPKKISLKGSDGKFYIMMCKPKDDLRKDCRLME
FNSLINKCLRKDAESRRRELHIRTYAVIPLNDECGIIEWVNNTAGLRPILTKLYKEKGVY
MTGKELRQCMLPKSAALSEKLKVFREFLLPRHPPIFHEWFLRTFPDPTSWYSSRSAYCRS
TAVMSMVGYILGLGDRHGENILFDSLTGECVHVDFNCLFNKGETFEVPEIVPFRLTHNMV
NGMGPMGTEGLFRRACEVTMRLMRDQREPLMSVLKTFLHDPLVEWSKPVKGHSKAPLNET
GEVVNEKAKTHVLDIEQRLQGVIKTRNRVTGLPLSIEGHVHYLIQEATDENLLCQMYLGW
TPYM

Figure 2.

MGDHGLELASMIPALRELGSATPEEYNTVVQKPRQILCQFIDRILTDVNVVALELVKKTD
AQPTSVMLLDFIQHIMKSSPLMFVNVNGSQGQNEAKDSCIEFSHWIITRLLRIAATPSCH
MLHKKICEVICSLLFLFKSKNPAIFGVLTRELLYLFEDLIYLHKRNAVGEVMEWPVVVSR
FLSRLDEHMGCLQPAPLQFMNVQNVEFIEVTLLMVLIHIVPTVFFRRQELLLWQIGCALL
EHGSPKIRSLAISLLTELFELGGLPAQPASTFFSLFLELLQHLVGMDADQLKLYEEPLSK
LLKTLFPFEAEAYRNIEPVYLNVLLEKLSVMFEDRVLMRLKSDLLKAALCHLLQYFLTFV
PAGYESALQVRKVYVTNICRALVDALGVQKHVGYLLGPFYAALKMESKEIIERIQCQAQQ
ENLS**GNNDEVSPKRRK**LSSSLSSYKKPSRQPEEIIHVDMDKKSILWNVLKQKAESLQISL
ECGTLKNSVAEALEGITVVLQLTALCTVHCSHQDMDGHNVKDHQHKYKKKPPVVVTWMSL
DFYTKVLKSCRSLLESVQKLELELVIDSMVRICDALMYMQVKSSFKDHVLEELCGMLSLP
WIYSYSDDNSLKMTTFATNLLPLSQRVWDSYSPQAQSKCVFLLTLFPRRIFLEWRTAVYN
WALKSSHEVIRASCVKGFFILLHQQNSCNQIPKMLVDRVKDDSDMVKKEFASVLGQLVCT
LHGMFYLSSSVEPCFEHMDLFSKNLKATSQHECSSSQVKASTCKPFLFLLTKNTPSPVKL
AFIDNLHHLCKHLDFQEDEREVKAVLGTLLNLMEDPDKDVRIAFSGNIKYILESLNSEDG
FVKELFVLRMKEAYTHAQIARNNELKDTLILTTGDIGRAAKGDLIPFALLHLLHCLLSKS
ASVSGAAYTEIRALVAAKSVKLQNFFSQYKKPICQFLVESLHSSQMTALPSAPCQSSEIR
KQDVAHHREMALNTLSEIANVFDFPDLNRFLTRTLQVLLPDLAAKASPAASALIRTLGKQ
LNVSRREILINNFKYIFSHLVCSCSKDELERALHYLKNETEIELGSLLRQDFQGLHNELL
LRIGEHYQQVFNGLSILASFASSDDPYQGPRDITSPELMADYLQPKLLGILAFFNMQLLS
SSVGIEDKKMALTSLMSLMKLMGPKHVSSVRVKMMTTLRTGLRFKDDFPELCCRAWDCFV
RCLDHAYLGPLLSHVIVALLPLIHMQPKETAAIFHYLIIENRDAVQDFLHEIYFLPDHPE
LEKIKAVLQEYRKETSETTDLQTTLQLSMKAIQHENVDVRIHALTSLKETLYKNQEKLIK
YATDSETVEPVISQLVTVILKGCQDANSQARLLCGECLGELGAIDPGRLDFSTTETQGKD
FTFVTGVEDLSFAYGLLMELTRAYLAYADNSRAQDSAAYAIQELLSIYDCREMQSNGPGY
QLWKRFPEHVREILEPHLNTRYKSSQKSTDWSGVTKPIYLSKLGNNFAEWSSSWAGYLIT
KVRDNLASKIFTCCSIMMKHDFKVTIYLLPHILVYVLLGCNQEDQQEVYAEIMAVLKHDE
QHAISTQDSASDLCQLSTQTVFSVLDHLTQWARHKFQALNAEKLAQNKPKGVSNVNFEDY
QSVTRFLDLIPQDTLAVASFRSKAYTRAVMHFESFITEKKQNIQKHLGFLQKLYAAMHEP
DGVAGVSAIRKAEPSLKEQILEHESIGLLRDATACYDRAIQLEPDQIIHYHGVVKSMLGL
GQLSTVITQVNGVHANRSEWTDELNTYRVEAAWKLSQWDLVENYLAADGKSTTWSVRLGQ
LLLSAKKRDTTTFYDTLKLVRAEQIVPLSAASFERGSYQRGYEFIVRLHMLCELEHSLKP
LFRKSPGDSCNEDSLNWGARLEMTQNSYRAKEPILALRRALLSLNKRPDYNEMVGECWLQ
SARVARKAGHHQTAYNALLNAGESRLAELYVERAKWLWSKGDVHQALIVLQKGVELCFPE
NKSPSESKHMLIHGRATLLVGRFMEETANFESNAVMKKYKDVTLFLPEWEDGHFYLAKYY
DKLMPMVTDNKMEKQGDLIRYIVLHFGRSLQYGNQFIYQSMPRMLSLWLDFGAKAYEWEK
GGRSDRLQMRNDLAKINSVLTEHTNRLAPYQFLTAFSQLISRICHSHDEVFVVLMEIIAK
VFLAYPQQAMWMMTAVSKSSYPMRVNRCKEILTKAIHMKKSLEKFVGDATRLTDKLLELC
NKSVDGSNSTLSMSTHFKMLKRLVEDPTFSEILIPLQSVMIPTLPSVLGAHANHDPFPGH
WAYLAGFDDVVEILSSLQKPK**KISLKGSDGKFY**IMMCKPKDDLRKDCRLMEFNSLINKSL
RKDAESRRRELHIRTYAVIPLNDECGIIEWVNNTAGLRPILTKIYKEKGVYMTGKELRQC
MLPKSAALSEKLKVFQELLLPRHPPVFHEWFLRTFPDPTSWYSSRSAYCRSTAVMSMVGY
ILGLGDRHGENILFDSFTGECVHVDFNCLFNKGETFEVPEIVPFRLTHNMVNGMGPMGTE
GLFRRACEVTLRLMRDQREPLMSVLKTFLHDPLVEGSKPVKGHSKAPLNETGEVVNEKAK
THVLDIEQRLQGVIKTRNRVTGLPLSIEGHVHYLIQEATDENLLCQMYLGWTPYM

Figure 3.


Total ATR


Phospho-ATR Ser428


Phospho-ATR Ser2317

1 2 3

1: Raw264.7 cells (Untreated)

2: Raw264.7 cells (UV-treated: 50mJ/cm^2, 30-minute recovery)
3: Raw264.7 cells (Nocodazole-treated: (50ng/ml, 16 hours)

SEQUENCE LISTING

<110> Cell Signaling Technology, Inc.
Livingstone, MARK
Ruan, Hong
Polakiewicz, Roberto

<120> Reagents for the Detection of PHosphorylated ATR Kinase (SER 428/SER 2317) and Uses Thereof

<130> CST-223 PCT

<150> USSN 60/700,979
<151> 2005-07-18

<160> 8

<170> PatentIn version 3.3

<210> 1
<211> 2644
<212> PRT
<213> Homo sapiens

<400> 1

Met Gly Glu His Gly Leu Glu Leu Ala Ser Met Ile Pro Ala Leu Arg
1 5 10 15

Glu Leu Gly Ser Ala Thr Pro Glu Glu Tyr Asn Thr Val Val Gln Lys
20 25 30

Pro Arg Gln Ile Leu Cys Gln Phe Ile Asp Arg Ile Leu Thr Asp Val
35 40 45

Asn Val Val Ala Val Glu Leu Val Lys Lys Thr Asp Ser Gln Pro Thr
50 55 60

Ser Val Met Leu Leu Asp Phe Ile Gln His Ile Met Lys Ser Ser Pro
65 70 75 80

Leu Met Phe Val Asn Val Ser Gly Ser His Glu Ala Lys Gly Ser Cys
85 90 95

Ile Glu Phe Ser Asn Trp Ile Ile Thr Arg Leu Leu Arg Ile Ala Ala
100 105 110

Thr Pro Ser Cys His Leu Leu His Lys Lys Ile Cys Glu Val Ile Cys
115 120 125

Ser Leu Leu Phe Leu Phe Lys Ser Lys Ser Pro Ala Ile Phe Gly Val
130 135 140

Leu Thr Lys Glu Leu Leu Gln Leu Phe Glu Asp Leu Val Tyr Leu His
145 150 155 160

Arg Arg Asn Val Met Gly His Ala Val Glu Trp Pro Val Val Met Ser
165 170 175

Arg Phe Leu Ser Gln Leu Asp Glu His Met Gly Tyr Leu Gln Ser Ala
180 185 190

```
Pro Leu Gln Leu Met Ser Met Gln Asn Leu Glu Phe Ile Glu Val Thr
        195                 200                 205

Leu Leu Met Val Leu Thr Arg Ile Ile Ala Ile Val Phe Phe Arg Arg
    210                 215                 220

Gln Glu Leu Leu Leu Trp Gln Ile Gly Cys Val Leu Leu Glu Tyr Gly
225                 230                 235                 240

Ser Pro Lys Ile Lys Ser Leu Ala Ile Ser Phe Leu Thr Glu Leu Phe
                245                 250                 255

Gln Leu Gly Gly Leu Pro Ala Gln Pro Ala Ser Thr Phe Phe Ser Ser
            260                 265                 270

Phe Leu Glu Leu Leu Lys His Leu Val Glu Met Asp Thr Asp Gln Leu
        275                 280                 285

Lys Leu Tyr Glu Glu Pro Leu Ser Lys Leu Ile Lys Thr Leu Phe Pro
    290                 295                 300

Phe Glu Ala Glu Ala Tyr Arg Asn Ile Glu Pro Val Tyr Leu Asn Met
305                 310                 315                 320

Leu Leu Glu Lys Leu Cys Val Met Phe Glu Asp Gly Val Leu Met Arg
                325                 330                 335

Leu Lys Ser Asp Leu Leu Lys Ala Ala Leu Cys His Leu Leu Gln Tyr
            340                 345                 350

Phe Leu Lys Phe Val Pro Ala Gly Tyr Glu Ser Ala Leu Gln Val Arg
        355                 360                 365

Lys Val Tyr Val Arg Asn Ile Cys Lys Ala Leu Leu Asp Val Leu Gly
    370                 375                 380

Ile Glu Val Asp Ala Glu Tyr Leu Leu Gly Pro Leu Tyr Ala Ala Leu
385                 390                 395                 400

Lys Met Glu Ser Met Glu Ile Ile Glu Glu Ile Gln Cys Gln Thr Gln
                405                 410                 415

Gln Glu Asn Leu Ser Ser Asn Ser Asp Gly Ile Ser Pro Lys Arg Arg
            420                 425                 430

Arg Leu Ser Ser Ser Leu Asn Pro Ser Lys Arg Ala Pro Lys Gln Thr
        435                 440                 445

Glu Glu Ile Lys His Val Asp Met Asn Gln Lys Ser Ile Leu Trp Ser
    450                 455                 460

Ala Leu Lys Gln Lys Ala Glu Ser Leu Gln Ile Ser Leu Glu Tyr Ser
465                 470                 475                 480
```

Gly Leu Lys Asn Pro Val Ile Glu Met Leu Glu Gly Ile Ala Val Val
485 490 495

Leu Gln Leu Thr Ala Leu Cys Thr Val His Cys Ser His Gln Asn Met
500 505 510

Asn Cys Arg Thr Phe Lys Asp Cys Gln His Lys Ser Lys Lys Lys Pro
515 520 525

Ser Val Val Ile Thr Trp Met Ser Leu Asp Phe Tyr Thr Lys Val Leu
530 535 540

Lys Ser Cys Arg Ser Leu Leu Glu Ser Val Gln Lys Leu Asp Leu Glu
545 550 555 560

Ala Thr Ile Asp Lys Val Val Lys Ile Tyr Asp Ala Leu Ile Tyr Met
565 570 575

Gln Val Asn Ser Ser Phe Glu Asp His Ile Leu Glu Asp Leu Cys Gly
580 585 590

Met Leu Ser Leu Pro Trp Ile Tyr Ser His Ser Asp Asp Gly Cys Leu
595 600 605

Lys Leu Thr Thr Phe Ala Ala Asn Leu Leu Thr Leu Ser Cys Arg Ile
610 615 620

Ser Asp Ser Tyr Ser Pro Gln Ala Gln Ser Arg Cys Val Phe Leu Leu
625 630 635 640

Thr Leu Phe Pro Arg Arg Ile Phe Leu Glu Trp Arg Thr Ala Val Tyr
645 650 655

Asn Trp Ala Leu Gln Ser Ser His Glu Val Ile Arg Ala Ser Cys Val
660 665 670

Ser Gly Phe Phe Ile Leu Leu Gln Gln Gln Asn Ser Cys Asn Arg Val
675 680 685

Pro Lys Ile Leu Ile Asp Lys Val Lys Asp Asp Ser Asp Ile Val Lys
690 695 700

Lys Glu Phe Ala Ser Ile Leu Gly Gln Leu Val Cys Thr Leu His Gly
705 710 715 720

Met Phe Tyr Leu Thr Ser Ser Leu Thr Glu Pro Phe Ser Glu His Gly
725 730 735

His Val Asp Leu Phe Cys Arg Asn Leu Lys Ala Thr Ser Gln His Glu
740 745 750

Cys Ser Ser Ser Gln Leu Lys Ala Ser Val Cys Lys Pro Phe Leu Phe
755 760 765

```
Leu Leu Lys Lys Lys Ile Pro Ser Pro Val Lys Leu Ala Phe Ile Asp
    770                 775                 780

Asn Leu His His Leu Cys Lys His Leu Asp Phe Arg Glu Asp Glu Thr
785                 790                 795                 800

Asp Val Lys Ala Val Leu Gly Thr Leu Leu Asn Leu Met Glu Asp Pro
                805                 810                 815

Asp Lys Asp Val Arg Val Ala Phe Ser Gly Asn Ile Lys His Ile Leu
            820                 825                 830

Glu Ser Leu Asp Ser Glu Asp Gly Phe Ile Lys Glu Leu Phe Val Leu
        835                 840                 845

Arg Met Lys Glu Ala Tyr Thr His Ala Gln Ile Ser Arg Asn Asn Glu
    850                 855                 860

Leu Lys Asp Thr Leu Ile Leu Thr Thr Gly Asp Ile Gly Arg Ala Ala
865                 870                 875                 880

Lys Gly Asp Leu Val Pro Phe Ala Leu Leu His Leu Leu His Cys Leu
                885                 890                 895

Leu Ser Lys Ser Ala Ser Val Ser Gly Ala Ala Tyr Thr Glu Ile Arg
            900                 905                 910

Ala Leu Val Ala Ala Lys Ser Val Lys Leu Gln Ser Phe Phe Ser Gln
        915                 920                 925

Tyr Lys Lys Pro Ile Cys Gln Phe Leu Val Glu Ser Leu His Ser Ser
    930                 935                 940

Gln Met Thr Ala Leu Pro Asn Thr Pro Cys Gln Asn Ala Asp Val Arg
945                 950                 955                 960

Lys Gln Asp Val Ala His Gln Arg Glu Met Ala Leu Asn Thr Leu Ser
                965                 970                 975

Glu Ile Ala Asn Val Phe Asp Phe Pro Asp Leu Asn Arg Phe Leu Thr
            980                 985                 990

Arg Thr Leu Gln Val Leu Leu Pro  Asp Leu Ala Ala Lys  Ala Ser Pro
        995                 1000                1005

Ala Ala  Ser Ala Leu Ile Arg  Thr Leu Gly Lys Gln  Leu Asn Val
    1010                1015                1020

Asn Arg  Arg Glu Ile Leu Ile  Asn Asn Phe Lys Tyr  Ile Phe Ser
    1025                1030                1035

His Leu  Val Cys Ser Cys Ser  Lys Asp Glu Leu Glu  Arg Ala Leu
    1040                1045                1050
```

```
His Tyr  Leu Lys Asn Glu Thr  Glu Ile Glu Leu Gly  Ser Leu Leu
    1055                 1060                 1065

Arg Gln  Asp Phe Gln Gly Leu  His Asn Glu Leu Leu  Leu Arg Ile
    1070                 1075                 1080

Gly Glu  His Tyr Gln Gln Val  Phe Asn Gly Leu Ser  Ile Leu Ala
    1085                 1090                 1095

Ser Phe  Ala Ser Ser Asp Asp  Pro Tyr Gln Gly Pro  Arg Asp Ile
    1100                 1105                 1110

Ile Ser  Pro Glu Leu Met Ala  Asp Tyr Leu Gln Pro  Lys Leu Leu
    1115                 1120                 1125

Gly Ile  Leu Ala Phe Phe Asn  Met Gln Leu Leu Ser  Ser Ser Val
    1130                 1135                 1140

Gly Ile  Glu Asp Lys Lys Met  Ala Leu Asn Ser Leu  Met Ser Leu
    1145                 1150                 1155

Met Lys  Leu Met Gly Pro Lys  His Val Ser Ser Val  Arg Val Lys
    1160                 1165                 1170

Met Met  Thr Thr Leu Arg Thr  Gly Leu Arg Phe Lys  Asp Asp Phe
    1175                 1180                 1185

Pro Glu  Leu Cys Cys Arg Ala  Trp Asp Cys Phe Val  Arg Cys Leu
    1190                 1195                 1200

Asp His  Ala Cys Leu Gly Ser  Leu Leu Ser His Val  Ile Val Ala
    1205                 1210                 1215

Leu Leu  Pro Leu Ile His Ile  Gln Pro Lys Glu Thr  Ala Ala Ile
    1220                 1225                 1230

Phe His  Tyr Leu Ile Ile Glu  Asn Arg Asp Ala Val  Gln Asp Phe
    1235                 1240                 1245

Leu His  Glu Ile Tyr Phe Leu  Pro Asp His Pro Glu  Leu Lys Lys
    1250                 1255                 1260

Ile Lys  Ala Val Leu Gln Glu  Tyr Arg Lys Glu Thr  Ser Glu Ser
    1265                 1270                 1275

Thr Asp  Leu Gln Thr Thr Leu  Gln Leu Ser Met Lys  Ala Ile Gln
    1280                 1285                 1290

His Glu  Asn Val Asp Val Arg  Ile His Ala Leu Thr  Ser Leu Lys
    1295                 1300                 1305

Glu Thr  Leu Tyr Lys Asn Gln  Glu Lys Leu Ile Lys  Tyr Ala Thr
    1310                 1315                 1320
```

```
Asp Ser  Glu Thr Val Glu Pro  Ile Ile Ser Gln Leu  Val Thr Val
     1325                 1330                 1335

Leu Leu  Lys Gly Cys Gln Asp  Ala Asn Ser Gln Ala  Arg Leu Leu
     1340                 1345                 1350

Cys Gly  Glu Cys Leu Gly Glu  Leu Gly Ala Ile Asp  Pro Gly Arg
     1355                 1360                 1365

Leu Asp  Phe Ser Thr Thr Glu  Thr Gln Gly Lys Asp  Phe Thr Phe
     1370                 1375                 1380

Val Thr  Gly Val Glu Asp Ser  Ser Phe Ala Tyr Gly  Leu Leu Met
     1385                 1390                 1395

Glu Leu  Thr Arg Ala Tyr Leu  Ala Tyr Ala Asp Asn  Ser Arg Ala
     1400                 1405                 1410

Gln Asp  Ser Ala Ala Tyr Ala  Ile Gln Glu Leu Leu  Ser Ile Tyr
     1415                 1420                 1425

Asp Cys  Arg Glu Met Glu Thr  Asn Gly Pro Gly His  Gln Leu Trp
     1430                 1435                 1440

Arg Arg  Phe Pro Glu His Val  Arg Glu Ile Leu Glu  Pro His Leu
     1445                 1450                 1455

Asn Thr  Arg Tyr Lys Ser Ser  Gln Lys Ser Thr Asp  Trp Ser Gly
     1460                 1465                 1470

Val Lys  Lys Pro Ile Tyr Leu  Ser Lys Leu Gly Ser  Asn Phe Ala
     1475                 1480                 1485

Glu Trp  Ser Ala Ser Trp Ala  Gly Tyr Leu Ile Thr  Lys Val Arg
     1490                 1495                 1500

His Asp  Leu Ala Ser Lys Ile  Phe Thr Cys Cys Ser  Ile Met Met
     1505                 1510                 1515

Lys His  Asp Phe Lys Val Thr  Ile Tyr Leu Leu Pro  His Ile Leu
     1520                 1525                 1530

Val Tyr  Val Leu Leu Gly Cys  Asn Gln Glu Asp Gln  Gln Glu Val
     1535                 1540                 1545

Tyr Ala  Glu Ile Met Ala Val  Leu Lys His Asp Asp  Gln His Thr
     1550                 1555                 1560

Ile Asn  Thr Gln Asp Ile Ala  Ser Asp Leu Cys Gln  Leu Ser Thr
     1565                 1570                 1575

Gln Thr  Val Phe Ser Met Leu  Asp His Leu Thr Gln  Trp Ala Arg
     1580                 1585                 1590
```

```
His Lys  Phe Gln Ala Leu Lys  Ala Glu Lys Cys Pro  His Ser Lys
     1595                 1600                 1605

Ser Asn  Arg Asn Lys Val Asp  Ser Met Val Ser Thr  Val Asp Tyr
     1610                 1615                 1620

Glu Asp  Tyr Gln Ser Val Thr  Arg Phe Leu Asp Leu  Ile Pro Gln
     1625                 1630                 1635

Asp Thr  Leu Ala Val Ala Ser  Phe Arg Ser Lys Ala  Tyr Thr Arg
     1640                 1645                 1650

Ala Val  Met His Phe Glu Ser  Phe Ile Thr Glu Lys  Lys Gln Asn
     1655                 1660                 1665

Ile Gln  Glu His Leu Gly Phe  Leu Gln Lys Leu Tyr  Ala Ala Met
     1670                 1675                 1680

His Glu  Pro Asp Gly Val Ala  Gly Val Ser Ala Ile  Arg Lys Ala
     1685                 1690                 1695

Glu Pro  Ser Leu Lys Glu Gln  Ile Leu Glu His Glu  Ser Leu Gly
     1700                 1705                 1710

Leu Leu  Arg Asp Ala Thr Ala  Cys Tyr Asp Arg Ala  Ile Gln Leu
     1715                 1720                 1725

Glu Pro  Asp Gln Ile Ile His  Tyr His Gly Val Val  Lys Ser Met
     1730                 1735                 1740

Leu Gly  Leu Gly Gln Leu Ser  Thr Val Ile Thr Gln  Val Asn Gly
     1745                 1750                 1755

Val His  Ala Asn Arg Ser Glu  Trp Thr Asp Glu Leu  Asn Thr Tyr
     1760                 1765                 1770

Arg Val  Glu Ala Ala Trp Lys  Leu Ser Gln Trp Asp  Leu Val Glu
     1775                 1780                 1785

Asn Tyr  Leu Ala Ala Asp Gly  Lys Ser Thr Thr Trp  Ser Val Arg
     1790                 1795                 1800

Leu Gly  Gln Leu Leu Leu Ser  Ala Lys Lys Arg Asp  Ile Thr Ala
     1805                 1810                 1815

Phe Tyr  Asp Ser Leu Lys Leu  Val Arg Ala Glu Gln  Ile Val Pro
     1820                 1825                 1830

Leu Ser  Ala Ala Ser Phe Glu  Arg Gly Ser Tyr Gln  Arg Gly Tyr
     1835                 1840                 1845

Glu Tyr  Ile Val Arg Leu His  Met Leu Cys Glu Leu  Glu His Ser
     1850                 1855                 1860
```

```
Ile Lys  Pro Leu Phe Gln His  Ser Pro Gly Asp Ser  Ser Gln Glu
    1865                 1870                 1875

Asp Ser  Leu Asn Trp Val Ala  Arg Leu Glu Met Thr  Gln Asn Ser
    1880                 1885                 1890

Tyr Arg  Ala Lys Glu Pro Ile  Leu Ala Leu Arg Arg  Ala Leu Leu
    1895                 1900                 1905

Ser Leu  Asn Lys Arg Pro Asp  Tyr Asn Glu Met Val  Gly Glu Cys
    1910                 1915                 1920

Trp Leu  Gln Ser Ala Arg Val  Ala Arg Lys Ala Gly  His His Gln
    1925                 1930                 1935

Thr Ala  Tyr Asn Ala Leu Leu  Asn Ala Gly Glu Ser  Arg Leu Ala
    1940                 1945                 1950

Glu Leu  Tyr Val Glu Arg Ala  Lys Trp Leu Trp Ser  Lys Gly Asp
    1955                 1960                 1965

Val His  Gln Ala Leu Ile Val  Leu Gln Lys Gly Val  Glu Leu Cys
    1970                 1975                 1980

Phe Pro  Glu Asn Glu Thr Pro  Pro Glu Gly Lys Asn  Met Leu Ile
    1985                 1990                 1995

His Gly  Arg Ala Met Leu Leu  Val Gly Arg Phe Met  Glu Glu Thr
    2000                 2005                 2010

Ala Asn  Phe Glu Ser Asn Ala  Ile Met Lys Lys Tyr  Lys Asp Val
    2015                 2020                 2025

Thr Ala  Cys Leu Pro Glu Trp  Glu Asp Gly His Phe  Tyr Leu Ala
    2030                 2035                 2040

Lys Tyr  Tyr Asp Lys Leu Met  Pro Met Val Thr Asp  Asn Lys Met
    2045                 2050                 2055

Glu Lys  Gln Gly Asp Leu Ile  Arg Tyr Ile Val Leu  His Phe Gly
    2060                 2065                 2070

Arg Ser  Leu Gln Tyr Gly Asn  Gln Phe Ile Tyr Gln  Ser Met Pro
    2075                 2080                 2085

Arg Met  Leu Thr Leu Trp Leu  Asp Tyr Gly Thr Lys  Ala Tyr Glu
    2090                 2095                 2100

Trp Glu  Lys Ala Gly Arg Ser  Asp Arg Val Gln Met  Arg Asn Asp
    2105                 2110                 2115

Leu Gly  Lys Ile Asn Lys Val  Ile Thr Glu His Thr  Asn Tyr Leu
    2120                 2125                 2130
```

```
Ala Pro  Tyr Gln Phe Leu Thr  Ala Phe Ser Gln Leu  Ile Ser Arg
     2135                 2140                 2145

Ile Cys  His Ser His Asp Glu  Val Phe Val Val Leu  Met Glu Ile
     2150                 2155                 2160

Ile Ala  Lys Val Phe Leu Ala  Tyr Pro Gln Gln Ala  Met Trp Met
     2165                 2170                 2175

Met Thr  Ala Val Ser Lys Ser  Ser Tyr Pro Met Arg  Val Asn Arg
     2180                 2185                 2190

Cys Lys  Glu Ile Leu Asn Lys  Ala Ile His Met Lys  Lys Ser Leu
     2195                 2200                 2205

Glu Lys  Phe Val Gly Asp Ala  Thr Arg Leu Thr Asp  Lys Leu Leu
     2210                 2215                 2220

Glu Leu  Cys Asn Lys Pro Val  Asp Gly Ser Ser Ser  Thr Leu Ser
     2225                 2230                 2235

Met Ser  Thr His Phe Lys Met  Leu Lys Lys Leu Val  Glu Glu Ala
     2240                 2245                 2250

Thr Phe  Ser Glu Ile Leu Ile  Pro Leu Gln Ser Val  Met Ile Pro
     2255                 2260                 2265

Thr Leu  Pro Ser Ile Leu Gly  Thr His Ala Asn His  Ala Ser His
     2270                 2275                 2280

Glu Pro  Phe Pro Gly His Trp  Ala Tyr Ile Ala Gly  Phe Asp Asp
     2285                 2290                 2295

Met Val  Glu Ile Leu Ala Ser  Leu Gln Lys Pro Lys  Lys Ile Ser
     2300                 2305                 2310

Leu Lys  Gly Ser Asp Gly Lys  Phe Tyr Ile Met Met  Cys Lys Pro
     2315                 2320                 2325

Lys Asp  Asp Leu Arg Lys Asp  Cys Arg Leu Met Glu  Phe Asn Ser
     2330                 2335                 2340

Leu Ile  Asn Lys Cys Leu Arg  Lys Asp Ala Glu Ser  Arg Arg Arg
     2345                 2350                 2355

Glu Leu  His Ile Arg Thr Tyr  Ala Val Ile Pro Leu  Asn Asp Glu
     2360                 2365                 2370

Cys Gly  Ile Ile Glu Trp Val  Asn Asn Thr Ala Gly  Leu Arg Pro
     2375                 2380                 2385

Ile Leu  Thr Lys Leu Tyr Lys  Glu Lys Gly Val Tyr  Met Thr Gly
     2390                 2395                 2400
```

```
Lys Glu  Leu Arg Gln Cys Met  Leu Pro Lys Ser Ala  Ala Leu Ser
    2405                 2410                 2415

Glu Lys  Leu Lys Val Phe Arg  Glu Phe Leu Leu Pro  Arg His Pro
    2420                 2425                 2430

Pro Ile  Phe His Glu Trp Phe  Leu Arg Thr Phe Pro  Asp Pro Thr
    2435                 2440                 2445

Ser Trp  Tyr Ser Ser Arg Ser  Ala Tyr Cys Arg Ser  Thr Ala Val
    2450                 2455                 2460

Met Ser  Met Val Gly Tyr Ile  Leu Gly Leu Gly Asp  Arg His Gly
    2465                 2470                 2475

Glu Asn  Ile Leu Phe Asp Ser  Leu Thr Gly Glu Cys  Val His Val
    2480                 2485                 2490

Asp Phe  Asn Cys Leu Phe Asn  Lys Gly Glu Thr Phe  Glu Val Pro
    2495                 2500                 2505

Glu Ile  Val Pro Phe Arg Leu  Thr His Asn Met Val  Asn Gly Met
    2510                 2515                 2520

Gly Pro  Met Gly Thr Glu Gly  Leu Phe Arg Arg Ala  Cys Glu Val
    2525                 2530                 2535

Thr Met  Arg Leu Met Arg Asp  Gln Arg Glu Pro Leu  Met Ser Val
    2540                 2545                 2550

Leu Lys  Thr Phe Leu His Asp  Pro Leu Val Glu Trp  Ser Lys Pro
    2555                 2560                 2565

Val Lys  Gly His Ser Lys Ala  Pro Leu Asn Glu Thr  Gly Glu Val
    2570                 2575                 2580

Val Asn  Glu Lys Ala Lys Thr  His Val Leu Asp Ile  Glu Gln Arg
    2585                 2590                 2595

Leu Gln  Gly Val Ile Lys Thr  Arg Asn Arg Val Thr  Gly Leu Pro
    2600                 2605                 2610

Leu Ser  Ile Glu Gly His Val  His Tyr Leu Ile Gln  Glu Ala Thr
    2615                 2620                 2625

Asp Glu  Asn Leu Leu Cys Gln  Met Tyr Leu Gly Trp  Thr Pro Tyr
    2630                 2635                 2640

Met
```

<210> 2
<211> 2644

<212> PRT
<213> Mus musculus

<400> 2

```
Met Gly Glu His Gly Leu Glu Leu Ala Ser Met Ile Pro Ala Leu Arg
1               5                  10                  15

Glu Leu Gly Ser Ala Thr Pro Glu Glu Tyr Asn Thr Val Val Gln Lys
            20                  25                  30

Pro Arg Gln Ile Leu Cys Gln Phe Ile Asp Arg Ile Leu Thr Asp Val
        35                  40                  45

Asn Val Val Ala Val Glu Leu Val Lys Lys Thr Asp Ser Gln Pro Thr
    50                  55                  60

Ser Val Met Leu Leu Asp Phe Ile Gln His Ile Met Lys Ser Ser Pro
65                  70                  75                  80

Leu Met Phe Val Asn Val Ser Gly Ser His Glu Ala Lys Gly Ser Cys
                85                  90                  95

Ile Glu Phe Ser Asn Trp Ile Ile Thr Arg Leu Leu Arg Ile Ala Ala
            100                 105                 110

Thr Pro Ser Cys His Leu Leu His Lys Lys Ile Cys Glu Val Ile Cys
        115                 120                 125

Ser Leu Leu Phe Leu Phe Lys Ser Lys Ser Pro Ala Ile Phe Gly Val
    130                 135                 140

Leu Thr Lys Glu Leu Leu Gln Leu Phe Glu Asp Leu Val Tyr Leu His
145                 150                 155                 160

Arg Arg Asn Val Met Gly His Ala Val Glu Trp Pro Val Val Met Ser
                165                 170                 175

Arg Phe Leu Ser Gln Leu Asp Glu His Met Gly Tyr Leu Gln Ser Ala
            180                 185                 190

Pro Leu Gln Leu Met Ser Met Gln Asn Leu Glu Phe Ile Glu Val Thr
        195                 200                 205

Leu Leu Met Val Leu Thr Arg Ile Ile Ala Ile Val Phe Phe Arg Arg
    210                 215                 220

Gln Glu Leu Leu Leu Trp Gln Ile Gly Cys Val Leu Leu Glu Tyr Gly
225                 230                 235                 240

Ser Pro Lys Ile Lys Ser Leu Ala Ile Ser Phe Leu Thr Glu Leu Phe
                245                 250                 255

Gln Leu Gly Gly Leu Pro Ala Gln Pro Ala Ser Thr Phe Phe Ser Ser
            260                 265                 270
```

Phe Leu Glu Leu Leu Lys His Leu Val Glu Met Asp Thr Asp Gln Leu
275 280 285

Lys Leu Tyr Glu Glu Pro Leu Ser Lys Leu Ile Lys Thr Leu Phe Pro
290 295 300

Phe Glu Ala Glu Ala Tyr Arg Asn Ile Glu Pro Val Tyr Leu Asn Met
305 310 315 320

Leu Leu Glu Lys Leu Cys Val Met Phe Glu Asp Gly Val Leu Met Arg
325 330 335

Leu Lys Ser Asp Leu Leu Lys Ala Ala Leu Cys His Leu Leu Gln Tyr
340 345 350

Phe Leu Lys Phe Val Pro Ala Gly Tyr Glu Ser Ala Leu Gln Val Arg
355 360 365

Lys Val Tyr Val Arg Asn Ile Cys Lys Ala Leu Leu Asp Val Leu Gly
370 375 380

Ile Glu Val Asp Ala Glu Tyr Leu Leu Gly Pro Leu Tyr Ala Ala Leu
385 390 395 400

Lys Met Glu Ser Met Glu Ile Ile Glu Glu Ile Gln Cys Gln Thr Gln
405 410 415

Gln Glu Asn Leu Ser Ser Asn Ser Asp Gly Ile Ser Pro Lys Arg Arg
420 425 430

Arg Leu Ser Ser Ser Leu Asn Pro Ser Lys Arg Ala Pro Lys Gln Thr
435 440 445

Glu Glu Ile Lys His Val Asp Met Asn Gln Lys Ser Ile Leu Trp Ser
450 455 460

Ala Leu Lys Gln Lys Ala Glu Ser Leu Gln Ile Ser Leu Glu Tyr Ser
465 470 475 480

Gly Leu Lys Asn Pro Val Ile Glu Met Leu Glu Gly Ile Ala Val Val
485 490 495

Leu Gln Leu Thr Ala Leu Cys Thr Val His Cys Ser His Gln Asn Met
500 505 510

Asn Cys Arg Thr Phe Lys Asp Cys Gln His Lys Ser Lys Lys Lys Pro
515 520 525

Ser Val Val Ile Thr Trp Met Ser Leu Asp Phe Tyr Thr Lys Val Leu
530 535 540

Lys Ser Cys Arg Ser Leu Leu Glu Ser Val Gln Lys Leu Asp Leu Glu
545 550 555 560

Ala Thr Ile Asp Lys Val Val Lys Ile Tyr Asp Ala Leu Ile Tyr Met
565 570 575

Gln Val Asn Ser Ser Phe Glu Asp His Ile Leu Glu Asp Leu Cys Gly
580 585 590

Met Leu Ser Leu Pro Trp Ile Tyr Ser His Ser Asp Asp Gly Cys Leu
595 600 605

Lys Leu Thr Thr Phe Ala Ala Asn Leu Leu Thr Leu Ser Cys Arg Ile
610 615 620

Ser Asp Ser Tyr Ser Pro Gln Ala Gln Ser Arg Cys Val Phe Leu Leu
625 630 635 640

Thr Leu Phe Pro Arg Arg Ile Phe Leu Glu Trp Arg Thr Ala Val Tyr
645 650 655

Asn Trp Ala Leu Gln Ser Ser His Glu Val Ile Arg Ala Ser Cys Val
660 665 670

Ser Gly Phe Phe Ile Leu Leu Gln Gln Gln Asn Ser Cys Asn Arg Val
675 680 685

Pro Lys Ile Leu Ile Asp Lys Val Lys Asp Asp Ser Asp Ile Val Lys
690 695 700

Lys Glu Phe Ala Ser Ile Leu Gly Gln Leu Val Cys Thr Leu His Gly
705 710 715 720

Met Phe Tyr Leu Thr Ser Ser Leu Thr Glu Pro Phe Ser Glu His Gly
725 730 735

His Val Asp Leu Phe Cys Arg Asn Leu Lys Ala Thr Ser Gln His Glu
740 745 750

Cys Ser Ser Ser Gln Leu Lys Ala Ser Val Cys Lys Pro Phe Leu Phe
755 760 765

Leu Leu Lys Lys Lys Ile Pro Ser Pro Val Lys Leu Ala Phe Ile Asp
770 775 780

Asn Leu His His Leu Cys Lys His Leu Asp Phe Arg Glu Asp Glu Thr
785 790 795 800

Asp Val Lys Ala Val Leu Gly Thr Leu Leu Asn Leu Met Glu Asp Pro
805 810 815

Asp Lys Asp Val Arg Val Ala Phe Ser Gly Asn Ile Lys His Ile Leu
820 825 830

Glu Ser Leu Asp Ser Glu Asp Gly Phe Ile Lys Glu Leu Phe Val Leu
835 840 845

```
Arg Met Lys Glu Ala Tyr Thr His Ala Gln Ile Ser Arg Asn Asn Glu
    850                 855                 860

Leu Lys Asp Thr Leu Ile Leu Thr Thr Gly Asp Ile Gly Arg Ala Ala
865                 870                 875                 880

Lys Gly Asp Leu Val Pro Phe Ala Leu Leu His Leu Leu His Cys Leu
                885                 890                 895

Leu Ser Lys Ser Ala Ser Val Ser Gly Ala Ala Tyr Thr Glu Ile Arg
            900                 905                 910

Ala Leu Val Ala Ala Lys Ser Val Lys Leu Gln Ser Phe Phe Ser Gln
        915                 920                 925

Tyr Lys Lys Pro Ile Cys Gln Phe Leu Val Glu Ser Leu His Ser Ser
    930                 935                 940

Gln Met Thr Ala Leu Pro Asn Thr Pro Cys Gln Asn Ala Asp Val Arg
945                 950                 955                 960

Lys Gln Asp Val Ala His Gln Arg Glu Met Ala Leu Asn Thr Leu Ser
                965                 970                 975

Glu Ile Ala Asn Val Phe Asp Phe Pro Asp Leu Asn Arg Phe Leu Thr
            980                 985                 990

Arg Thr Leu Gln Val Leu Leu Pro  Asp Leu Ala Ala Lys  Ala Ser Pro
        995                 1000                1005

Ala Ala  Ser Ala Leu Ile Arg  Thr Leu Gly Lys Gln  Leu Asn Val
    1010                1015                1020

Asn Arg  Arg Glu Ile Leu Ile  Asn Asn Phe Lys Tyr  Ile Phe Ser
    1025                1030                1035

His Leu  Val Cys Ser Cys Ser  Lys Asp Glu Leu Glu  Arg Ala Leu
    1040                1045                1050

His Tyr  Leu Lys Asn Glu Thr  Glu Ile Glu Leu Gly  Ser Leu Leu
    1055                1060                1065

Arg Gln  Asp Phe Gln Gly Leu  His Asn Glu Leu Leu  Leu Arg Ile
    1070                1075                1080

Gly Glu  His Tyr Gln Gln Val  Phe Asn Gly Leu Ser  Ile Leu Ala
    1085                1090                1095

Ser Phe  Ala Ser Ser Asp Asp  Pro Tyr Gln Gly Pro  Arg Asp Ile
    1100                1105                1110

Ile Ser  Pro Glu Leu Met Ala  Asp Tyr Leu Gln Pro  Lys Leu Leu
    1115                1120                1125
```

Gly Ile Leu Ala Phe Phe Asn Met Gln Leu Leu Ser Ser Ser Val
1130 1135 1140

Gly Ile Glu Asp Lys Lys Met Ala Leu Asn Ser Leu Met Ser Leu
1145 1150 1155

Met Lys Leu Met Gly Pro Lys His Val Ser Ser Val Arg Val Lys
1160 1165 1170

Met Met Thr Thr Leu Arg Thr Gly Leu Arg Phe Lys Asp Asp Phe
1175 1180 1185

Pro Glu Leu Cys Cys Arg Ala Trp Asp Cys Phe Val Arg Cys Leu
1190 1195 1200

Asp His Ala Cys Leu Gly Ser Leu Leu Ser His Val Ile Val Ala
1205 1210 1215

Leu Leu Pro Leu Ile His Ile Gln Pro Lys Glu Thr Ala Ala Ile
1220 1225 1230

Phe His Tyr Leu Ile Ile Glu Asn Arg Asp Ala Val Gln Asp Phe
1235 1240 1245

Leu His Glu Ile Tyr Phe Leu Pro Asp His Pro Glu Leu Lys Lys
1250 1255 1260

Ile Lys Ala Val Leu Gln Glu Tyr Arg Lys Glu Thr Ser Glu Ser
1265 1270 1275

Thr Asp Leu Gln Thr Thr Leu Gln Leu Ser Met Lys Ala Ile Gln
1280 1285 1290

His Glu Asn Val Asp Val Arg Ile His Ala Leu Thr Ser Leu Lys
1295 1300 1305

Glu Thr Leu Tyr Lys Asn Gln Glu Lys Leu Ile Lys Tyr Ala Thr
1310 1315 1320

Asp Ser Glu Thr Val Glu Pro Ile Ile Ser Gln Leu Val Thr Val
1325 1330 1335

Leu Leu Lys Gly Cys Gln Asp Ala Asn Ser Gln Ala Arg Leu Leu
1340 1345 1350

Cys Gly Glu Cys Leu Gly Glu Leu Gly Ala Ile Asp Pro Gly Arg
1355 1360 1365

Leu Asp Phe Ser Thr Thr Glu Thr Gln Gly Lys Asp Phe Thr Phe
1370 1375 1380

Val Thr Gly Val Glu Asp Ser Ser Phe Ala Tyr Gly Leu Leu Met
1385 1390 1395

```
Glu Leu  Thr Arg Ala Tyr Leu  Ala Tyr Ala Asp Asn  Ser Arg Ala
    1400                 1405                 1410

Gln Asp  Ser Ala Ala Tyr Ala  Ile Gln Glu Leu Leu  Ser Ile Tyr
    1415                 1420                 1425

Asp Cys  Arg Glu Met Glu Thr  Asn Gly Pro Gly His  Gln Leu Trp
    1430                 1435                 1440

Arg Arg  Phe Pro Glu His Val  Arg Glu Ile Leu Glu  Pro His Leu
    1445                 1450                 1455

Asn Thr  Arg Tyr Lys Ser Ser  Gln Lys Ser Thr Asp  Trp Ser Gly
    1460                 1465                 1470

Val Lys  Lys Pro Ile Tyr Leu  Ser Lys Leu Gly Ser  Asn Phe Ala
    1475                 1480                 1485

Glu Trp  Ser Ala Ser Trp Ala  Gly Tyr Leu Ile Thr  Lys Val Arg
    1490                 1495                 1500

His Asp  Leu Ala Ser Lys Ile  Phe Thr Cys Cys Ser  Ile Met Met
    1505                 1510                 1515

Lys His  Asp Phe Lys Val Thr  Ile Tyr Leu Leu Pro  His Ile Leu
    1520                 1525                 1530

Val Tyr  Val Leu Leu Gly Cys  Asn Gln Glu Asp Gln  Gln Glu Val
    1535                 1540                 1545

Tyr Ala  Glu Ile Met Ala Val  Leu Lys His Asp Asp  Gln His Thr
    1550                 1555                 1560

Ile Asn  Thr Gln Asp Ile Ala  Ser Asp Leu Cys Gln  Leu Ser Thr
    1565                 1570                 1575

Gln Thr  Val Phe Ser Met Leu  Asp His Leu Thr Gln  Trp Ala Arg
    1580                 1585                 1590

His Lys  Phe Gln Ala Leu Lys  Ala Glu Lys Cys Pro  His Ser Lys
    1595                 1600                 1605

Ser Asn  Arg Asn Lys Val Asp  Ser Met Val Ser Thr  Val Asp Tyr
    1610                 1615                 1620

Glu Asp  Tyr Gln Ser Val Thr  Arg Phe Leu Asp Leu  Ile Pro Gln
    1625                 1630                 1635

Asp Thr  Leu Ala Val Ala Ser  Phe Arg Ser Lys Ala  Tyr Thr Arg
    1640                 1645                 1650

Ala Val  Met His Phe Glu Ser  Phe Ile Thr Glu Lys  Lys Gln Asn
    1655                 1660                 1665
```

```
Ile Gln  Glu His Leu Gly Phe  Leu Gln Lys Leu Tyr  Ala Ala Met
    1670                 1675                 1680

His Glu  Pro Asp Gly Val Ala  Gly Val Ser Ala Ile  Arg Lys Ala
    1685                 1690                 1695

Glu Pro  Ser Leu Lys Glu Gln  Ile Leu Glu His Glu  Ser Leu Gly
    1700                 1705                 1710

Leu Leu  Arg Asp Ala Thr Ala  Cys Tyr Asp Arg Ala  Ile Gln Leu
    1715                 1720                 1725

Glu Pro  Asp Gln Ile Ile His  Tyr His Gly Val Val  Lys Ser Met
    1730                 1735                 1740

Leu Gly  Leu Gly Gln Leu Ser  Thr Val Ile Thr Gln  Val Asn Gly
    1745                 1750                 1755

Val His  Ala Asn Arg Ser Glu  Trp Thr Asp Glu Leu  Asn Thr Tyr
    1760                 1765                 1770

Arg Val  Glu Ala Ala Trp Lys  Leu Ser Gln Trp Asp  Leu Val Glu
    1775                 1780                 1785

Asn Tyr  Leu Ala Ala Asp Gly  Lys Ser Thr Thr Trp  Ser Val Arg
    1790                 1795                 1800

Leu Gly  Gln Leu Leu Leu Ser  Ala Lys Lys Arg Asp  Ile Thr Ala
    1805                 1810                 1815

Phe Tyr  Asp Ser Leu Lys Leu  Val Arg Ala Glu Gln  Ile Val Pro
    1820                 1825                 1830

Leu Ser  Ala Ala Ser Phe Glu  Arg Gly Ser Tyr Gln  Arg Gly Tyr
    1835                 1840                 1845

Glu Tyr  Ile Val Arg Leu His  Met Leu Cys Glu Leu  Glu His Ser
    1850                 1855                 1860

Ile Lys  Pro Leu Phe Gln His  Ser Pro Gly Asp Ser  Ser Gln Glu
    1865                 1870                 1875

Asp Ser  Leu Asn Trp Val Ala  Arg Leu Glu Met Thr  Gln Asn Ser
    1880                 1885                 1890

Tyr Arg  Ala Lys Glu Pro Ile  Leu Ala Leu Arg Arg  Ala Leu Leu
    1895                 1900                 1905

Ser Leu  Asn Lys Arg Pro Asp  Tyr Asn Glu Met Val  Gly Glu Cys
    1910                 1915                 1920

Trp Leu  Gln Ser Ala Arg Val  Ala Arg Lys Ala Gly  His His Gln
    1925                 1930                 1935
```

```
Thr Ala  Tyr Asn Ala Leu Leu  Asn Ala Gly Glu Ser  Arg Leu Ala
    1940                 1945                 1950

Glu Leu  Tyr Val Glu Arg Ala  Lys Trp Leu Trp Ser  Lys Gly Asp
    1955                 1960                 1965

Val His  Gln Ala Leu Ile Val  Leu Gln Lys Gly Val  Glu Leu Cys
    1970                 1975                 1980

Phe Pro  Glu Asn Glu Thr Pro  Pro Glu Gly Lys Asn  Met Leu Ile
    1985                 1990                 1995

His Gly  Arg Ala Met Leu Leu  Val Gly Arg Phe Met  Glu Glu Thr
    2000                 2005                 2010

Ala Asn  Phe Glu Ser Asn Ala  Ile Met Lys Lys Tyr  Lys Asp Val
    2015                 2020                 2025

Thr Ala  Cys Leu Pro Glu Trp  Glu Asp Gly His Phe  Tyr Leu Ala
    2030                 2035                 2040

Lys Tyr  Tyr Asp Lys Leu Met  Pro Met Val Thr Asp  Asn Lys Met
    2045                 2050                 2055

Glu Lys  Gln Gly Asp Leu Ile  Arg Tyr Ile Val Leu  His Phe Gly
    2060                 2065                 2070

Arg Ser  Leu Gln Tyr Gly Asn  Gln Phe Ile Tyr Gln  Ser Met Pro
    2075                 2080                 2085

Arg Met  Leu Thr Leu Trp Leu  Asp Tyr Gly Thr Lys  Ala Tyr Glu
    2090                 2095                 2100

Trp Glu  Lys Ala Gly Arg Ser  Asp Arg Val Gln Met  Arg Asn Asp
    2105                 2110                 2115

Leu Gly  Lys Ile Asn Lys Val  Ile Thr Glu His Thr  Asn Tyr Leu
    2120                 2125                 2130

Ala Pro  Tyr Gln Phe Leu Thr  Ala Phe Ser Gln Leu  Ile Ser Arg
    2135                 2140                 2145

Ile Cys  His Ser His Asp Glu  Val Phe Val Val Leu  Met Glu Ile
    2150                 2155                 2160

Ile Ala  Lys Val Phe Leu Ala  Tyr Pro Gln Gln Ala  Met Trp Met
    2165                 2170                 2175

Met Thr  Ala Val Ser Lys Ser  Ser Tyr Pro Met Arg  Val Asn Arg
    2180                 2185                 2190

Cys Lys  Glu Ile Leu Asn Lys  Ala Ile His Met Lys  Lys Ser Leu
    2195                 2200                 2205
```

Glu Lys Phe Val Gly Asp Ala Thr Arg Leu Thr Asp Lys Leu Leu
2210 2215 2220

Glu Leu Cys Asn Lys Pro Val Asp Gly Ser Ser Ser Thr Leu Ser
2225 2230 2235

Met Ser Thr His Phe Lys Met Leu Lys Lys Leu Val Glu Glu Ala
2240 2245 2250

Thr Phe Ser Glu Ile Leu Ile Pro Leu Gln Ser Val Met Ile Pro
2255 2260 2265

Thr Leu Pro Ser Ile Leu Gly Thr His Ala Asn His Ala Ser His
2270 2275 2280

Glu Pro Phe Pro Gly His Trp Ala Tyr Ile Ala Gly Phe Asp Asp
2285 2290 2295

Met Val Glu Ile Leu Ala Ser Leu Gln Lys Pro Lys Lys Ile Ser
2300 2305 2310

Leu Lys Gly Ser Asp Gly Lys Phe Tyr Ile Met Met Cys Lys Pro
2315 2320 2325

Lys Asp Asp Leu Arg Lys Asp Cys Arg Leu Met Glu Phe Asn Ser
2330 2335 2340

Leu Ile Asn Lys Cys Leu Arg Lys Asp Ala Glu Ser Arg Arg Arg
2345 2350 2355

Glu Leu His Ile Arg Thr Tyr Ala Val Ile Pro Leu Asn Asp Glu
2360 2365 2370

Cys Gly Ile Ile Glu Trp Val Asn Asn Thr Ala Gly Leu Arg Pro
2375 2380 2385

Ile Leu Thr Lys Leu Tyr Lys Glu Lys Gly Val Tyr Met Thr Gly
2390 2395 2400

Lys Glu Leu Arg Gln Cys Met Leu Pro Lys Ser Ala Ala Leu Ser
2405 2410 2415

Glu Lys Leu Lys Val Phe Arg Glu Phe Leu Leu Pro Arg His Pro
2420 2425 2430

Pro Ile Phe His Glu Trp Phe Leu Arg Thr Phe Pro Asp Pro Thr
2435 2440 2445

Ser Trp Tyr Ser Ser Arg Ser Ala Tyr Cys Arg Ser Thr Ala Val
2450 2455 2460

Met Ser Met Val Gly Tyr Ile Leu Gly Leu Gly Asp Arg His Gly
2465 2470 2475

Glu Asn Ile Leu Phe Asp Ser Leu Thr Gly Glu Cys Val His Val
2480 2485 2490

Asp Phe Asn Cys Leu Phe Asn Lys Gly Glu Thr Phe Glu Val Pro
2495 2500 2505

Glu Ile Val Pro Phe Arg Leu Thr His Asn Met Val Asn Gly Met
2510 2515 2520

Gly Pro Met Gly Thr Glu Gly Leu Phe Arg Arg Ala Cys Glu Val
2525 2530 2535

Thr Met Arg Leu Met Arg Asp Gln Arg Glu Pro Leu Met Ser Val
2540 2545 2550

Leu Lys Thr Phe Leu His Asp Pro Leu Val Glu Trp Ser Lys Pro
2555 2560 2565

Val Lys Gly His Ser Lys Ala Pro Leu Asn Glu Thr Gly Glu Val
2570 2575 2580

Val Asn Glu Lys Ala Lys Thr His Val Leu Asp Ile Glu Gln Arg
2585 2590 2595

Leu Gln Gly Val Ile Lys Thr Arg Asn Arg Val Thr Gly Leu Pro
2600 2605 2610

Leu Ser Ile Glu Gly His Val His Tyr Leu Ile Gln Glu Ala Thr
2615 2620 2625

Asp Glu Asn Leu Leu Cys Gln Met Tyr Leu Gly Trp Thr Pro Tyr
2630 2635 2640

Met

<210> 3
<211> 15
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (8)..(8)
<223> PHOSPHORYLATION: Serine at position 8 is phosphorylated

<400> 3

Ser Ser Asn Ser Asp Gly Ile Ser Pro Lys Arg Arg Arg Leu Ser
1 5 10 15

<210> 4
<211> 15
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (8)..(8)
<223> PHOSPHORYLATION: Serine at position 8 is phosphorylated

<400> 4

Lys Lys Ile Ser Leu Lys Gly Ser Asp Gly Lys Phe Tyr Ile Met
1 5 10 15

<210> 5
<211> 13
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (7)..(7)
<223> PHOSPHORYLATION: Serine at position 7 is phosphorylated

<400> 5

Ser Asn Ser Asp Gly Ile Ser Pro Lys Arg Arg Arg Leu
1 5 10

<210> 6
<211> 11
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (6)..(6)
<223> PHOSPHORYLATION: Serine at position 6 is phosphorylated

<400> 6

Ile Ser Leu Lys Gly Ser Asp Gly Lys Phe Tyr
1 5 10

<210> 7
<211> 30
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (10)..(10)
<223> PHOSPHORYLATION: Serine at position 10 is phosphorylated

<400> 7

Asn Leu Ser Ser Asn Ser Asp Gly Ile Ser Pro Lys Arg Arg Arg Leu
1 5 10 15

Ser Ser Ser Leu Asn Pro Ser Lys Arg Ala Pro Lys Gln Thr
20 25 30

<210> 8
<211> 32
<212> PRT
<213> Homo sapiens

<220>
<221> MOD_RES
<222> (20)..(20)
<223> PHOSPHORYLATION: Serine at position 20 i s phosphorylateed

<400> 8

Asp Met Val Glu Ile Leu Ala Ser Leu Gln Lys Pro Lys Lys Ile Ser
1 5 10 15

Leu Lys Gly Ser Asp Gly Lys Phe Tyr Ile Met Met Cys Lys Pro Lys
20 25 30