

Molecular Interaction Map of the Mammalian Cell Cycle Control and DNA Repair Systems

Kurt W. Kohn*

Laboratory of Molecular Pharmacology, Division of Basic Sciences, National Cancer Institute, Bethesda, Maryland 20892

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Eventually to understand the integrated function of the cell cycle regulatory network, we must organize the known interactions in the form of a diagram, map, and/or database. A diagram convention was designed capable of unambiguous representation of networks containing multi-protein complexes, protein modifications, and enzymes that are substrates of other enzymes. To facilitate linkage to a database, each molecular species is symbolically represented only once in each diagram. Molecular species can be located on the map by means of indexed grid coordinates. Each interaction is referenced to an annotation list where pertinent information and references can be found. Parts of the network are grouped into functional subsystems. The map shows how multiprotein complexes could assemble and function at gene promoter sites and at sites of DNA damage. It also portrays the richness of connections between the p53-Mdm2 subsystem and other parts of the network.

INTRODUCTION

The complexity of the molecular interactions implicated in cell regulatory networks challenges human comprehension. Current diagrams of molecular interactions are often ambiguous or incomplete. The preparation of more comprehensive regulatory network diagrams is both difficult and urgent. The difficulties are not due merely to the large number of

reactions, because this is a feature also of the familiar metabolic pathway diagrams. They are due rather to complexities that rarely occur in classical pathway diagrams, such as multisubunit complexes, protein modifications, enzymes that are modified by other enzymes, and protein domains whose function is regulated by other domains of the same molecule. The present work describes and applies a diagram method designed to cope with these kinds of complexity.

Why do we need molecular interaction maps? First, it is often difficult to keep in mind all of the known interactions that may be pertinent to a particular experimental or theoretical question, and a molecular interaction map can be used in much the same way as a road map or electronic circuit diagram. Second, molecular interaction maps can suggest new interpretations or questions for experiment. Third, the act of preparing a molecular interaction map imposes a discipline of logic and critique to the formulation of functional models. Finally, the diagram convention provides a shorthand for recording complicated findings or hypotheses.

Another kind of difficulty in preparing useful maps is the incompleteness and uncertainty of knowledge, as well as the limited scope of applicability of some interactions. An important aspect of molecular interaction maps, as described here, is that they are linked to an annotation list that summarizes current information relevant to particular interactions and provides references. A molecular interaction map can therefore function also as a review. The maps can be updated interactively via the Internet and thus can provide a current summary of an area.

* Corresponding author. E-mail address: kohnk@dc37a.nci.nih.gov. Abbreviations used: ARF, alternate reading frame of the ink4a locus (murine p19, human p14); ATM, ataxia telangiectasia mutated gene/protein; BRCT, homologous to breast cancer protein 1 C-terminal module; CAK, Cdk-activating kinase (consisting of Cyclin H, Cdk7, and p36MAT1); C/EBP, CCAAT/enhancer-binding proteins; CK, casein kinase; CTD, C-terminal domain; Cyc, Cyclin; DNA-PK, DNA-dependent protein kinase (catalytic unit, not including Ku); DHFR, dihydrofolate reductase; DPase, DNA polymerase; ERCC, excision repair cross-complementing; dsDNA, double-stranded DNA; E2, recognition sites in promoters that bind E2F transcription factors; FEN, flap endonuclease; HDAC, histone deacetylase; HMG, high-mobility group protein; Ku, Ku70:Ku80/86 heterodimer (same as XRCC5 and 6); IR, ionizing radiation; NER, nucleotide excision repair; PARP, poly-(ADP-ribose) polymerase; Plk, mammalian polo-like kinase; PCNA, proliferating cell nuclear antigen; RPA, replication protein A (comprising subunits p70RPA1, p34RPA2, and p14RPA3); RPase, RNA polymerase; SH, Src homology; ssb, DNA single-strand breaks; ssDNA, single-stranded DNA; TAF, TBP-associated factor; TBP, TATA-binding protein; TFIIH, transcription factor IIH; UV, UV light; XPA, xeroderma pigmentosum complementation group A gene/protein (similarly for XPB, etc.); XRCC1, x-ray repair cross-complementing gene/protein 1.

The current work describes the mapping conventions and uses them to build a molecular interaction map of the circuitry that governs the mammalian cell cycle and DNA repair machinery. Updated and corrected versions of the map will be accessible at the internet address discovery.nci.nih.gov.

MAP CONVENTIONS

Desirable Characteristics of Molecular Interaction Maps

A major consideration in the design of the diagram conventions was to facilitate tracing all the known interactions of any given molecular species. Accordingly, each molecular species should ideally appear only once in a diagram, and all interactions involving that species should emanate from a single symbolic construct. A second major consideration was a concise method to represent multimolecular complexes. Multimer proteins are common components of regulatory systems and sometimes function in large-scale multimolecular assemblies. Therefore, an extensible representation of such complexes was a fundamental requirement. A third major consideration was the representation of protein modifications, such as phosphorylations. One must be able to represent various modifications of a protein by unique graphical constructs. Meeting these goals simultaneously is a significant challenge.

Additionally, one must be able to show the actions or effects of each molecular species or interaction, including enzyme action and stimulation and inhibition of activity or binding. Often there are many interaction or modification sites having diverse effects on function. The potential number of modification-multimerization combinations is staggering, and we have barely begun to explore this vast domain experimentally. Representation of all of these possible combinations in a single diagram is obviously impractical. Nonetheless, it is important to be able to represent any combinations that may be significant.

Symbols and Rules

Because each molecular species in a diagram ideally should appear only once, interactions must be indicated by several types of lines connecting the species. The different types of interaction lines are distinguished by different kinds of arrowheads or other line endings, as summarized in Figure 1.

Multimolecular Complexes

Noncovalent binding between molecular species is indicated by lines terminated at both ends by barbed arrowheads. Thus noncovalent binding in most cases is represented symmetrically. In some cases, however, it is useful to distinguish a protein that has a receptor site from a protein that donates a peptide binding to the site, for example the binding of an N-terminal peptide of p53 to a pocket in Mdm2 (Kussie *et al.*, 1996). The receptor end of an interaction line can be represented by a double-barbed arrowhead (Figure 1). This notation can serve to indicate targets of opportunity for pharmacological intervention. Noncovalent binding is generally assumed to be reversible; when binding is unusually tight, the interaction line can be drawn heavier.

Having defined an intermolecular binding symbol, we next need a representation of the complex itself. This is accomplished by placing a small filled circle (or "node") on the connecting line. An action of the complex can then be represented by an appropriate type of line emanating from the node. See, for example, the enzymatic action line emanating from the node representing CycD:Cdk4 in Figure 5 (reaction 5), enzymatic action being indicated by the open circle at the end of the line, in accord with the symbol definition table (Figure 1).

Multiple actions of a complex can be depicted conveniently by using multiple nodes on the same line; each node then refers to exactly the same complex. See, for example, the two occurrences of node a in Figure 3.

Thus only the monomolecular species are indicated by name, and the identity of the complexes is determined by tracing the connecting lines back to the monomolecular units.

To represent alternative or competitive binding of different proteins at the same site, the lines from the competing proteins are merged before connecting to the site. Figure 2 illustrates the conciseness and flexibility of this representation. Each possible dimer (a, c, e, or g), or dimer pair for a given monomer (b, d, or f), is defined by specific placement of a node. Effects specific to any combination of interactions therefore can be represented unambiguously. For example, the actions of Cdk1 that occur regardless of whether the partner is Cyclin A or B would be indicated by an action line emanating from node f.

An important feature of the line-and-node representation is that it is extensible. Binding interactions involving a node are represented in the same way as binding interactions involving a monomer. To see how this works, consider the example in Figure 3, which represents the interactions of E2F1, DP1, pRb, and an E2 promoter element. Each of the naturally occurring molecular combinations of these monomers is indicated by a node, the promoter element being treated like a monomer species. The two filled circles labeled a, being on the same line segment, represent exactly the same molecular species, namely E2F1:DP1. The other node representations are as follows: b, E2F1:DP1:pRb; c, E2F1:DP1 bound to promoter element E2; and d, E2F1:DP1:pRb bound to E2. Also indicated is transcriptional stimulation or inhibition occurring when the promoter element is occupied by E2F1:DP1 (node c) or E2F1:DP1:pRb (node d), respectively. (See Figure 1 for definitions of stimulation and inhibition symbols. Also, note that connecting lines may change in direction, for example, with right-angle bends, and that crossing lines do not affect each other.) Using this type of scheme, most multimolecular contingencies can readily be depicted.

A complex containing multiple copies of the same monomer can be represented concisely by use of a ditto symbol, consisting of an isolated filled circle at the end of a single connecting line. See, for example, the representation of a homodimer in the lower part of Figure 1. The notation can be extended to higher homopolymers, as illustrated in Figure 4 for tetramerization of p53: three nodes are placed side by side to denote the three additional copies that, together with the identified monomer, make up the tetramer. This example shows the requirement of p53 tetramerization for

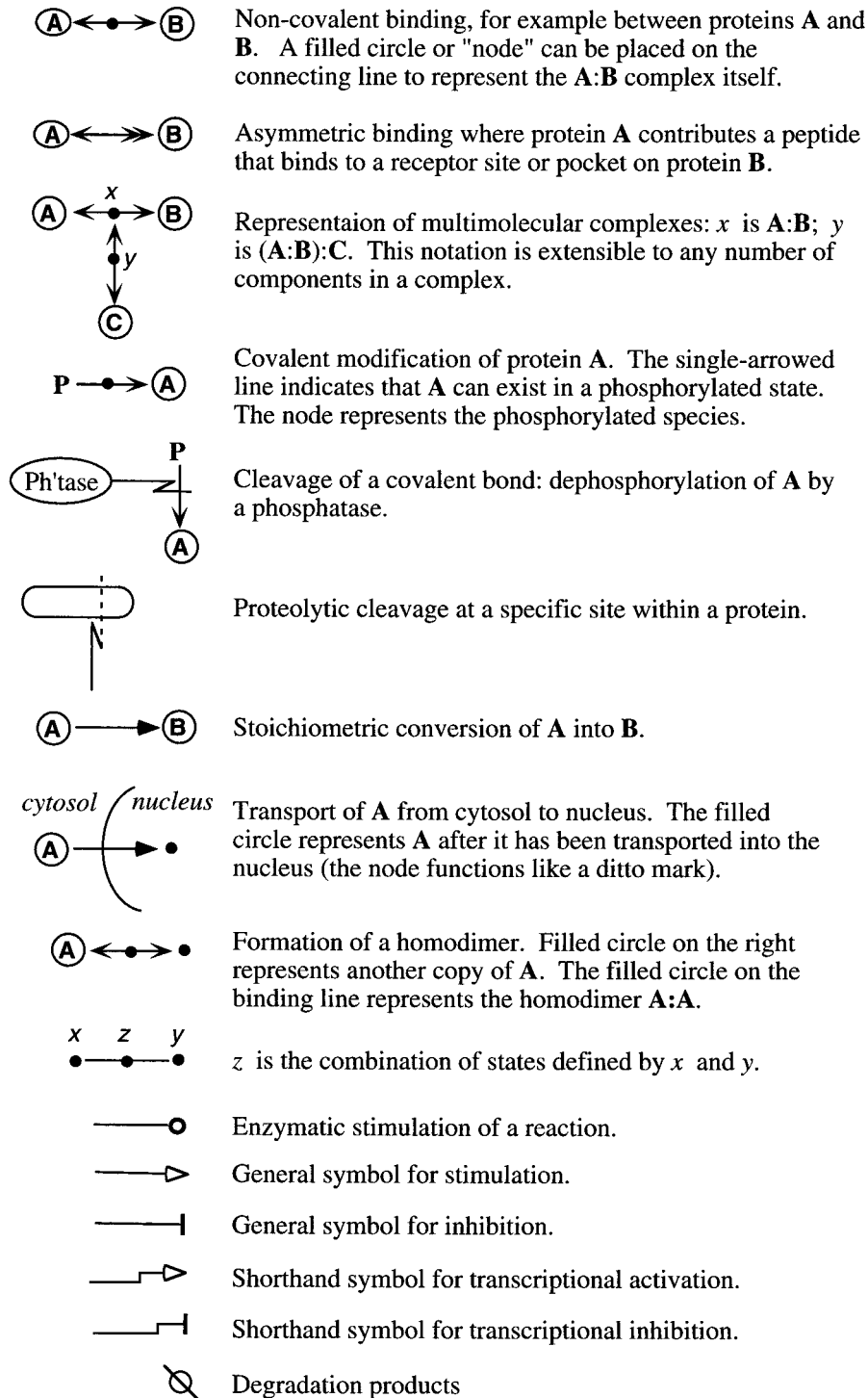


Figure 1. Summary of symbols.

binding to promoter elements and for phosphorylation of p53 at Ser15.

A given protein often can form stable complexes simultaneously with two or more different protein molecules. These are of special interest because the interactions can extend to form large-scale, functionally integrated multiprotein assemblies. When there is an extensive chain of interactions, however, it is often unknown how local interactions in 2705

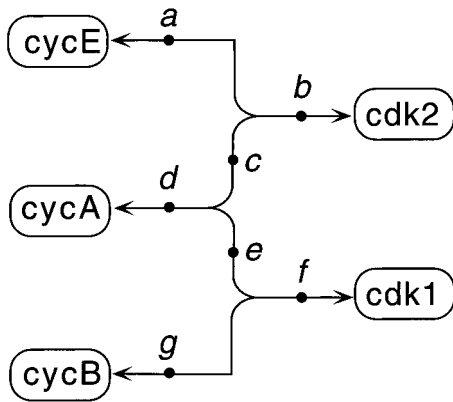


Figure 2. Concise representation of alternative binding modes. Example: heterodimers formed by Cyclins E, A, and B binding to Cdk1 or 2. (a) CycE:Cdk2; (b) Cdk2 complexed with either CycE or CycA; (c) CycA:Cdk2; (d) CycA complexed with either Cdk2 or Cdk1; (e) CycA:Cdk1; (f) Cdk1 complexed with either CycA or CycB. Note that a, c, d, e, and g each lie on a unique connector line, and each represents a unique heterodimer. Nodes b, d, and f, on the other hand, represent dimer combinations. This notation greatly simplifies the representation of multiple alternative interactions: for example, the interactions of p21, p27, or p57 with various Cyclin: Cdk dimers in Figure 6A. A formal rule, required to avoid ambiguity, is that lines representing alternative interactions must join at an acute angle.

ferent parts of the chain affect each other. The map conventions allow the binary interactions to be depicted without specifying all of the possible influences between different parts of a chain of interactions while at the same time specifying those influences that are known.

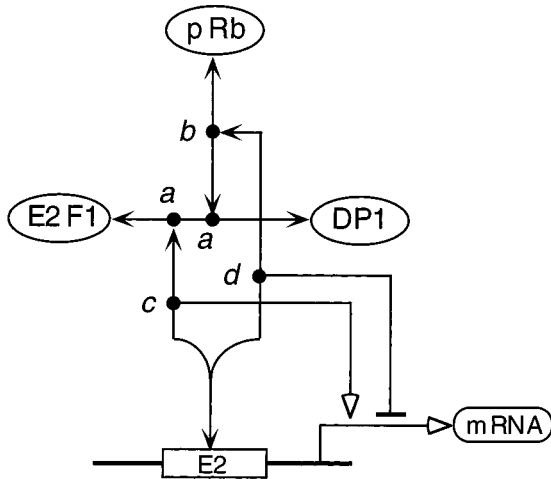


Figure 3. Representation of multimolecular complexes: stimulatory and inhibitory complexes of E2F1, DP1, and pRb. (a) E2F1:DP1 dimer; (b) E2F1:DP1:pRb trimer; (c) E2F1:DP1 bound to promoter element E2 (transcriptional activation shown); (d) E2F1:DP1:pRb bound to E2 (transcriptional inhibition shown). Note that the promoter element can be occupied either by E2F1:DP1 or by E2F1:DP1:pRb (alternative binding represented by interaction lines joined at an acute angle).

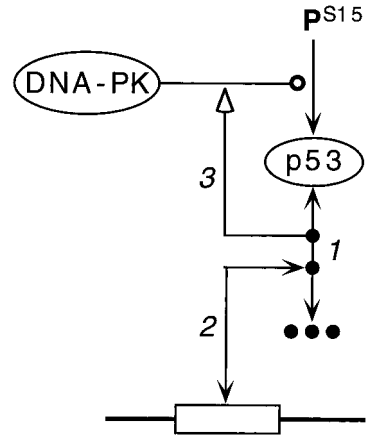


Figure 4. Concise representation of homopolymers: formation and effects of p53 homotetramer. (1) The three additional copies of p53 monomer required to make up the tetramer are represented by three nodes placed side by side and linked to the identified p53 monomer; a node placed internally on this line represents the homotetramer itself. (2) p53 tetramer can bind to promoter element. (3) Tetramerization stimulates (or is required for) phosphorylation of p53 Ser15 by DNA-PK.

Covalent Modifications

Most regulatory proteins are subject to a multiplicity of modifications, especially phosphorylations, that alter function. This presents a severe challenge to any diagram method. The difficulty is further increased when combinations of phosphorylations must be considered.

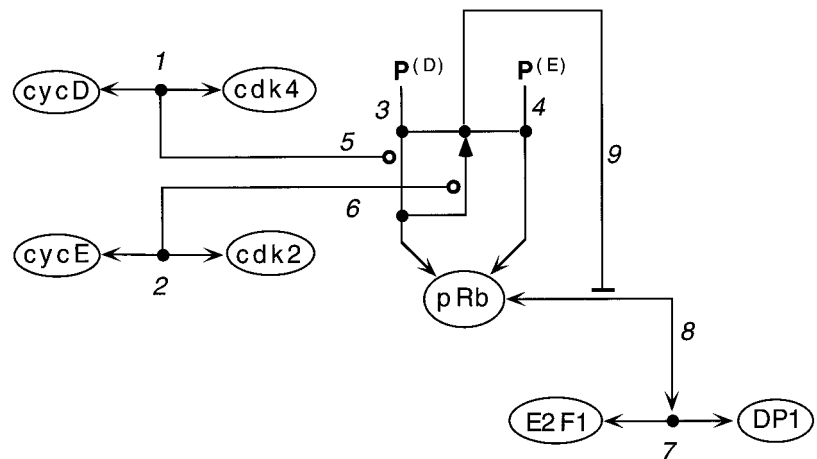
A phosphorylation (or other covalent modification) is represented by a line with a single barbed arrowhead that points to the modified protein (Figure 1). The multiplicity of phosphorylations and acetylations of p53 are included in the comprehensive diagram, Figure 6B. The modifications are arrayed along the length of the elongated, pill-shaped p53 outline from the N terminus on the left to the C terminus on the right. The amino acid positions of the modification sites are indicated. A node on a single-barb-arrowed line represents the protein modified at that site. The effects of a given modification on intra- or intermolecular actions are indicated by interaction lines emerging from the node.

Although the effects of multiple modifications on one another are largely unknown, some important interactions among modifications within the same protein molecule have been defined. To represent combinations of modifications, we need additional symbols. We use a nonarrowed connecting line to represent joint modifications; a node on such a connecting line represents the protein having the combined modifications. An example of this situation is provided by the phosphorylation states of pRb (Figure 5). pRb is multiply phosphorylated by CycD:Cdk4 and by CycE:Cdk2, but several of the sites differ. It seems that phosphorylation by CycD:Cdk4 is required before CycE:Cdk2 can phosphorylate its specific sites, and that both kinases are required to fully impair the inhibitory binding of pRb to E2F:DP complexes.

Multidomain Structures

Regulatory proteins often are composed of structural domains having different functions. This multidomain organi-

Figure 5. Phosphorylation control of pRb: illustration of the use of conjunction symbols to denote protein modification combinations. (1) Cyclin D binds Cdk4; the filled circle (node) on the line represents the CycD:Cdk4 complex itself. (2) Similarly for Cyclin E and Cdk2. (3) The single-headed line linking P^(D) to pRb represents phosphorylation of pRb at sites kinased by CycD:Cdk4; a node on this line represents pRb-P^(D) [the P^(D)-phosphorylated form of pRb]. (4) Similarly for pRb phosphorylated at sites, P^(E), kinased by CycE:Cdk2. (5) CycD:Cdk4 phosphorylates pRb at sites P^(D). (6) CycE:Cdk2 acts on pRb-P^(D), generating fully phosphorylated pRb; the line with the filled arrowhead indicates stoichiometric conversion of pRb-P^(D) to the fully phosphorylated form, which is represented by the node on the nonarrowed line connecting the pRb-P^(D) and pRb-P^(E) nodes. (7) E2F1 binds DP1. (8) pRb binds to E2F1:DP1. (9) Fully phosphorylated pRb cannot bind to E2F1:DP1. Thus dissociation of pRb from E2F1:DP1 requires hyperphosphorylation by Cyclin E:Cdk2, which in turn requires previous phosphorylation by Cyclin D:Cdk4/6 (Zarakowska and Mittnacht, 1997; Lundberg and Weinberg, 1998).



zation can integrate interactions with other molecules or communicate functions within a given protein molecule. To depict functional localization within a protein molecule, a horizontal, pill-shaped outline is used rather than an oval. Localized interactions or modifications proceeding from the N terminus on the left to the C terminus on the right are marked along the upper and/or lower borders of the pill shape (e.g., p53 in Figure 6B). When the locale of an interaction is unknown, it is marked at either end of the pill shape.

MOLECULAR INTERACTION MAP

Figure 6 presents a comprehensive molecular interaction map of regulators of cell cycle and DNA repair processes. The current map, however, is limited to events in the mammalian cell nucleus. Because of limits on what can legibly be formatted onto a journal page, the map is divided into two parts. Figure 6A maps the interactions involving E2F, pRb, Cyclin, and Cdk family members, their activators and inhibitors, as well as some important interactions with other components. Figure 6B focuses on the p53-Mdm2 subsystem and on subsystems related to DNA repair. Molecular components are grouped in putative subsystems according to mutual interactions or functional coherence. It remains to be determined whether subsystems can be identified on the basis of objective criteria.

The monomolecular species included in Figure 6 are listed alphabetically in an index (Table 1), which gives grid coordinates to help locate each species.

Some of the interaction patterns shown in the map deserve special comment. References are cited in the annotation list and can be found using the identifiers marked on the interaction lines in the map. These identifiers are italicized letter-number combinations, which are used also in the following text to specify particular interactions on the map.

We are concerned here primarily with molecular interactions rather than biological effects. The latter may eventually be understood as emerging from the former.

The E2F-pRb Box

The possible occupancy states of an E2F recognition element in a promoter comprise a multiplicity of complex patterns. The method of representing these patterns was shown by the simplified example in Figure 3. The simplest arrangement would be an E2F:DP heterodimer bound to an E2 element (E5). (The italicized letter-number combinations identify particular interactions in Figure 6 and refer to entries in the annotation list where references are cited.) Because promoters generally have two or more E2F recognition elements, however, the actual situation even in this case may be far from simple. Most E2F family proteins can activate transcription (E6) by way of a transactivation domain. E2F-6, however, has instead a transcription repressor domain (E9). E2F:DP heterodimers (other than those of E2F-6) can tether pRb family proteins onto promoters, thereby converting a potential gene activator into a repressor (E7). E2F in fact more often functions to repress rather than activate.

E2F proteins (as heterodimers with a DP protein [E1]) have individual binding preferences for pRb family members. The E2F-pRb box shows that pRb prefers E2F-1-, -2-, or -3-containing heterodimers (E2) (with lesser interaction with E2F-4), p107 binds only E2F-4 heterodimers, and p130 can bind heterodimers of E2F-4 or 5 (E3,4). All of these complexes can bind and repress E2F recognition elements (E6). E2F-6, however, does not bind any pRb family protein. E2F-1, -2, and -3 are marked together as a unit on the map, because of their mutual preference for pRb. Although there are significant functional distinctions between them, the molecular bases for these differences is not known. No major functional differences among different DP family members have been defined.

A further level of complexity arises from the ability of some E2F complexes to bind other transcription factors, such as Sp1, and to synergize the transcriptional activation.

Another mode of regulation arises from the ability of histone deacetylase (HDAC1) to bind to pRb:E2F:DP-type complexes (E13). HDAC1 could deactivate transcription that has been enhanced by histone acetylation (H1) and thus

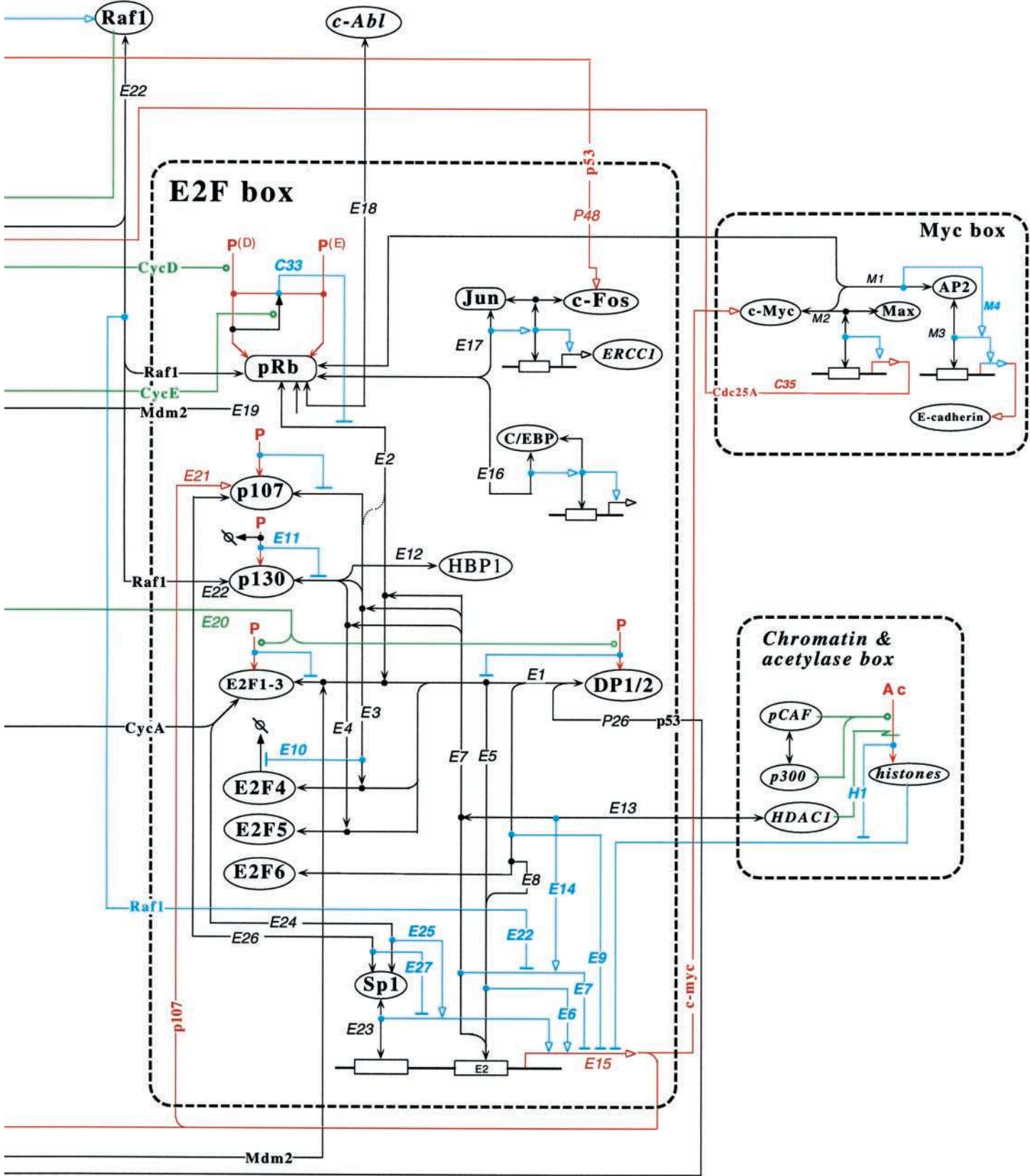
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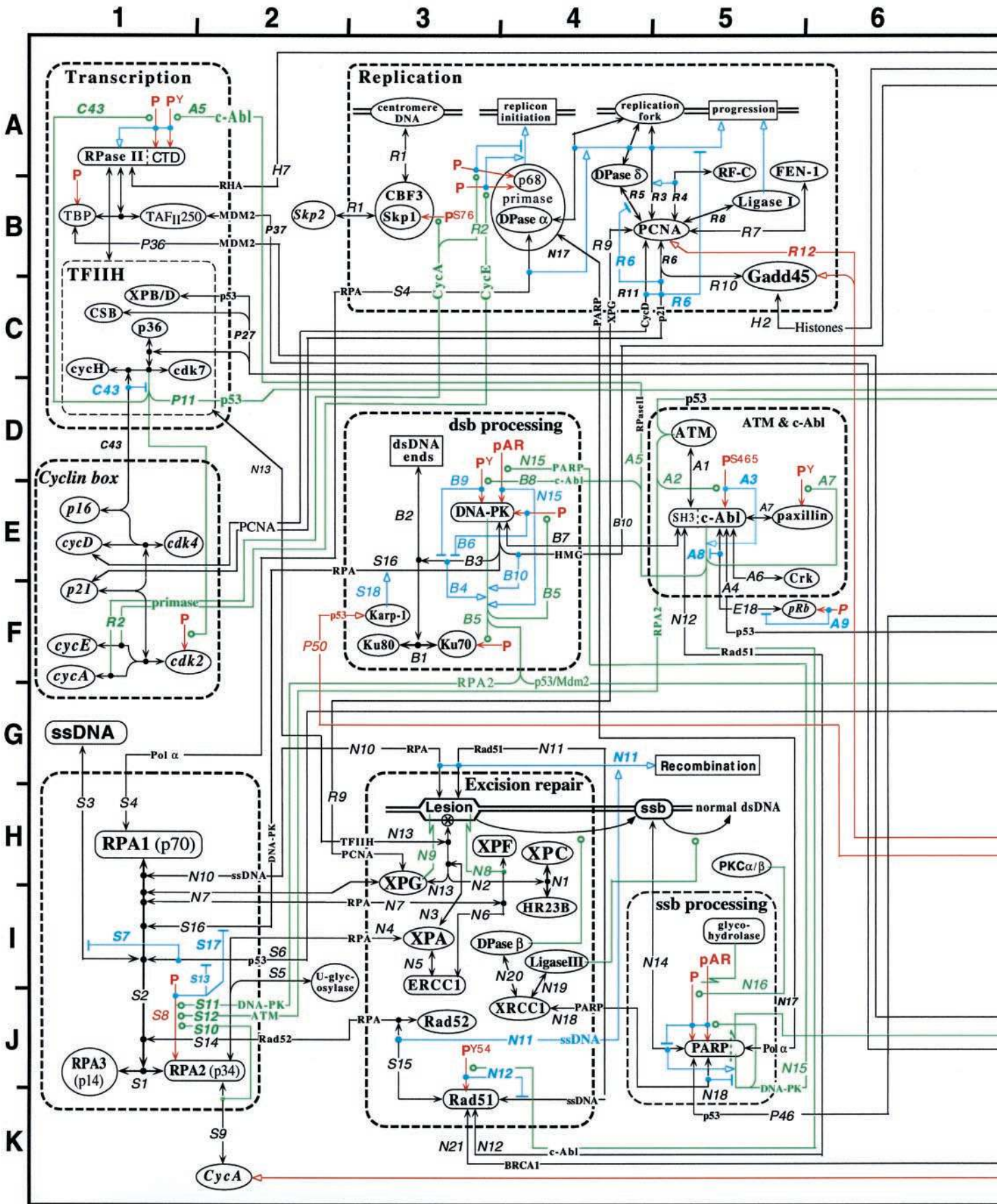
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could contribute to gene down-regulation by pRb family proteins.

pRb sometimes functions as a gene activator rather than repressor. For example, it can activate the Jun family and CCAAT/enhancer-binding protein (C/EBP) transcription factors (E16,17). The detailed mechanism by which this occurs is not known.

Taken together, the interactions noted on the map add up to perhaps 20 different possible states of an individual E2F recognition element.

The interaction capabilities of the constituent proteins are subject to modulation by phosphorylation, protein binding, and regulated degradation. In addition, regulated nuclear-cytoplasmic transport is emerging as an important process. Translocations can be represented as indicted in Figure 1 but, because of space limitations, have been omitted from the current version of the map. pRb is subject to different sets of multiple phosphorylation by CycD:Cdk4/6 (C31) and cycE:Cdk2 (C32). The manner of depiction of these phosphorylations and their effects was explained in Figure 5. Only the fully phosphorylated pRb is impaired with respect to E2F binding (C33). The E2F binding of p107 and p130 is also inhibited by phosphorylation (E11). Phosphorylation of E2F and/or DP by CycA:Cdk2 (which forms stable complexes with E2F-1) (E20) inhibits the E2F-DP interaction and could serve to turn off E2F function when cycA accumulates late in S phase.

pRb family proteins may also be inhibited by binding to other proteins, such as Raf-1 (E22). This may be one of the logical connections, suggested by current work, which may communicate signals from the cell surface to the cell cycle control circuitry.

The Cyclin-Cdk Box

The activity of Cdks is intricately regulated. To begin with, Cdk activity requires binding to a Cyclin. The map shows Cdk4 or Cdk6 (which have the same molecular interactions) binding to CycD (C3), Cdk2 binding to Cyclins E or A (C4), and Cdk1 (also known as Cdc2) binding to Cyclins A or B (C5). (The subtypes of CycB and CycD are not differentiated here.)

A second class of controls on Cdks are stimulatory and inhibitory phosphorylations, which are controlled by several kinases and phosphatases noted on the map. All Cdks are activated by phosphorylation of Thr160 (or 161), carried out by CycH:Cdk7 (C14), which functions also as a constituent

of the transcription factor IIIH (TFIIH) complex. Cdk1, and to some extent other Cdks, can be inhibited by phosphorylations corresponding to Thr14 and/or Tyr15 (C17,19,20). These sites are phosphorylated by Wee1 or Myt1 (C16). In the case of Cdk1, these inhibitory phosphorylations are removed by dual-action phosphatase Cdc25C, which is in turn activated by phosphorylations (C18) introduced by mammalian polo-like kinase 1 (Plk1) (C37) and/or Cdk1 (C36). Cdc25C can be phosphorylated at Ser216 by Chk1 (C38) or C-TAK1 (C39). Ser216 phosphorylation generates a binding site for 14-3-3, and this binding inhibits the phosphatase (C40).

A positive feedback loop that can be traced on the map consists of just two components: Cdc25C and CycB:Cdk1. Cdc25C is activated by hyperphosphorylation (C18); activated Cdc25C dephosphorylates Thr14 and Tyr15 of Cdk1, thereby removing the inhibitory effect of these phosphates on the kinase (C17) and increasing the activating phosphorylation of Cdc25C (C36). This positive feedback could help produce switch-like behavior and may operate in the G2 to M cell cycle phase transition.

A third class of controls acts through the binding of specific Cyclin:Cdk inhibitors, including p16ink4a, p21cip1, p27kip1, and p57kip2. p16ink4a inhibits by binding Cdk4/6 in competition with cycD (C8). p21cip1, p27kip1, and p57kip2 can bind Cyclin complexes of Cdk4/6 and Cdk2 (C7,23). There may be an additional complication, however, because p21 can stabilize and enhance the activity of cycD:Cdk4 when a single p21 molecule is bound but can inhibit the same activity when a second p21 molecule binds to the complex (C22). p27 can be phosphorylated by the kinase it inhibits, CycE:Cdk2 (C21). This seemingly paradoxical relationship might be due to intermolecular action of an active CycE:Cdk2 on an inactive CycE:Cdk2:p27 complex.

The Cyclin:Cdk system can interact with elements of the DNA replication and repair systems through binding of p21 (R6) or Cyclin D (R11) to proliferating cell nuclear antigen (PCNA). This action may also involve Gadd45, which can bind simultaneously to p21 (C34) and PCNA (R10). p21, PCNA, and Gadd45 are all transcriptionally activated by p53 (P43,44).

The p53-Mdm2 Box

The map shows the remarkable richness of p53 interconnections and the diversity of functionally determinative p53 modifications. Eleven phosphorylation or acetylation sites (or groups of sites) for which functionality has been surmised are shown. If all of these could occur independently, there would be ~2000 possible modification states of p53 monomers. Some interdependent modifications have been noted: phosphorylation of Thr18 requires previous phosphorylation of Ser15 (P3) (Appella, personal communication); acetylation of Lys320 requires tetramer structure of p53 and is inhibited by phosphorylation of Ser378 (P23) (Sakaguchi and Appella, personal communication). Other dependencies certainly exist, some perhaps having major functional impact, whereas many could have subtle quantitative effects, which may or may not convey a selective evolutionary advantage. Nevertheless, p53-expressing cells may contain hundreds of different modification states of p53 monomers.

Figure 6 (previous 4 pages). Molecular interaction map of the regulatory network that controls the mammalian cell cycle and DNA repair systems. The map is presented in two parts: A focuses on the E2F-pRb and Cyclin:Cdk subsystems and functionally related interactions; B focuses on the p53-Mdm2 and DNA repair subsystem and functionally related interactions. Some duplication between the two parts of the maps was unavoidable but was noted by using italicized species or subsystem names to indicate that their interactions are shown in greater detail on the other part of the map. Although interaction symbols are fully defined by the conventions listed in Figure 1, the following colors were used to enhance readability: black, binding interactions and stoichiometric conversions; red, covalent modifications and gene expression; green, enzyme actions; blue, stimulations and inhibitions. See APPENDIX for annotations.

Table 1. Index of monomolecular species

Species	Location	Species	Location
14-3-3	A2K, B11E	Mdm2	<i>A1I</i> , B7I
Abl	A7A, B5E	Myc	A9C
AP2	A10C	Myt1	A4J
APC	A3I	p107	A7E
ATM	B5D	p130	A7F
Bax	B10H	p14ARF, see p19ARF	
BRCA1	B10F	p16	A4B , <i>B1E</i>
Caspase 3	B7K	p19ARF	A6I , B8G
CBF3	A1G, B3B	p21Cip1	A3E , B1F , <i>BB11H</i>
Cdc25A	A4C	p27	A3E
Cdc25C	A4H	p300	<i>A10H</i> , B10B
Cdc2, see Cdk1		p36MAT1	B1C
Cdk1	A4I	p53	<i>A1H</i> , B8E
Cdk2	A4G, <i>B1F</i> , <i>B9B</i> , <i>B10D</i>	p57	A3F
Cdk4/6	A4D, <i>B1E</i>	p68	<i>A1F</i> , B4B
Cdk7	A2C, B1C	PARP	B5J
C/EBP	A8E	Paxillin	B5E
Chk1	A5H	pCAF	<i>A10G</i> , B10B
CK1 δ/κ	<i>B7B</i>	PCNA	<i>A1E</i> , B5B
CK2	<i>B7C</i>	PKC	<i>B7C</i>
Cks1	A5I	PKC α/β	B5H
Crk	B5E	Plk1	A3J
CSB	B1C	Pol α	<i>A1F</i>
C-TAK1	A5H	pRb	<i>A7D</i> , <i>B5F</i> , <i>B6J</i> , <i>B10E</i>
Cyclin A	A3H, <i>B1F</i> , <i>B2K</i> , <i>B9B</i>	Primase	<i>A1F</i> , B4B
Cyclin B	A3H	Rad51	B3K
Cyclin D	A3D, <i>B1E</i>	Rad52	B3J
Cyclin E	A3F, <i>B1F</i> , <i>B10D</i>	Raf1	A6A
Cyclin H	A1C, B1C	Ras	A5A
DMP1	A3C, <i>B6G</i>	Rb, see pRb	
DNA-PK	B3E	Replication fork	B4A
DP1/2	A9G, <i>B6F</i> , <i>B6I</i> , <i>B10D</i>	RF-C	B5A
DPase α	B4B	Rb, see pRb	
DPase β	B4I	RHA	B10D
DPase δ	B4A	RPA	<i>A1E</i>
dsDNA ends	B3D	RPA1	B1H
E2F1,2,3	A7G, <i>B6I</i> , <i>B10D</i>	RPA2	B2J
E2F4	A7H	RPA3	B1J
E2F5	A7H	RFase II	B1A
E2F6	A7H	Skp1	<i>A1G</i> , B3B
E-cadherin	A10D	Skp2	A3G , <i>B2B</i>
ERCC1	<i>A9D</i> , B3I	SL1	A1B
FEN-1	B5A	Sp1	A7J
Fos	A8C, <i>B11I</i>	ssb	B5H
Gadd45	A3D, B5B	ssDNA	B1G , <i>B9G</i>
HBP1	A8F	TAFII250	B1B
HDAC1	<i>A10H</i> , B11C	TBP	B1B
Histones	<i>A10H</i> , B11B	TFIIH	B1B
HMG	B11C	U-glycosylase	B2I
HR23B	B4I	Weel	A3J
JNK	<i>B7B</i>	XPA	B3I
Jun	A8C	XPB	B1C
Karp-1	B3F	XPC	B4H
Ku70	B3F	XPD	B1C
Ku80	B3F	XPF	B4F
Ligase I	B5B	XRCC1	B4J
Ligase III	B4I		
MAPK	<i>B7B</i>		
MAT1, see p36MAT1			
Max	<i>A10C</i>		

Bold letters refer to maps A or B of Figure 6, A or B, respectively. The number-letter combination that follows the bold letter is the location on the grid. Italics refer to locations where the interactions of the molecular species are included in abbreviated form only.

Some p53 modifications and interactions are especially notable. Ser15 appears to be the site of phosphorylation responses to DNA damage signals communicated by way of the kinases ataxia telangiectasia mutated gene/protein (ATM) (P2) and DNA-dependent protein kinase (DNA-PK) (P6). Phosphorylation of Ser18 or Ser20 prevents stable binding to Mdm2 (P5), thus abrogating the Mdm2-mediated inhibition (P29) and degradation (P31) of p53. Because these sites are located within the region required for Mdm2 binding, it is plausible that their phosphorylation could inhibit this interaction. p53 forms a stable complex with p300 (P25), as a result of which p300 acetylates p53 Lys382 (P21). This acetylation, as well as the acetylation of Lys320 by PCAF (P23), enhances the sequence-specific binding of p53 to promoters, probably indirectly by inhibiting nonspecific DNA binding (P22). A similar mechanism of enhanced promoter binding (P16) may occur as a result of binding of the p53 C-terminal region to 14-3-3 (P13), which requires 14-3-3 to be dimerized (P14).

p53 can bind to a number of proteins that are involved in DNA repair functions, cell cycle control, or general control functions. In approximate order of binding location from N to C termini of p53, these include the following: Mdm2 (which has a pocket that binds a p53 N-terminal peptide) (P28); p300 C-terminal region (P25); DP1 (P26); poly(ADP-ribose) polymerase (PARP) (P46); c-Abl (A4); replication protein A (RPA) (S6); high-mobility group protein (HMG) (P52); TFIIH constituent helicases xeroderma pigmentosum complementation group B (XPB) and XPD and DNA repair protein CSB (P27); p19ARF (P40); p300 N-terminal region (P33); BRCA1 (P47); and 14-3-3 (P13). Some of these interactions (p300, BRCA1, and 14-3-3) stimulate and some (Mdm2, PARP, and RPA) inhibit the transcriptional activity of p53. (Stimulations may be indirect: 14-3-3 may block nonspecific binding of p53 to DNA [P16]; p300 may do the same consequential to acetylation of K382 [P21,22].)

p53 can form homotetramers and must be in tetramer form for sequence-specific DNA binding and transcriptional activation. The map shows the dependencies relating to tetramers; tetramerization is stimulated by phosphorylation of Ser392, and this enhancement can be inhibited by phosphorylation of Ser315 (P17). The ability to form tetramers is further modulated by other modifications and protein interactions. Influence on tetramerization may be how p53 transcriptional activity is stimulated by binding BRCA1 and inhibited by binding Mdm2, PARP, or RPA. This could be the major mechanism of regulation of p53 transcriptional activity. The activation of p53 seems to be exquisitely controlled by a large number of determinative inputs. The transition to active tetramers could be very sharp because of a possible fourth-power dependence on the concentration of tetramerization-competent monomers.

Mdm2 is an intimate part of the p53 control system. Mdm2 contains a pocket that binds a p53 N-terminal peptide (P28). Mdm2 binding blocks the transcriptional activation domain of p53 (P29) and is instrumental in p53 degradation (P31). p53, in turn, transcriptionally up-regulates Mdm2, probably forming a negative feedback loop. Mdm2 can itself activate some genes, such as Cyclin A (P37). In addition to binding p53, Mdm2 reportedly binds E2F1:DP1 (P35), pRb (P35), TATA-binding protein (TBP) (P36), TBP-associated factor II250 (TAF_{II}250) (P37), p19ARF (P34), and p300 (P33).

Some of these interactions may compete for the same Mdm2 site, as may be the case for p19ARF and p300. Binding to p53 is abrogated by phosphorylation of Mdm2 on Ser17, perhaps through the kinase activity of DNA-PK (P49).

p19ARF, an alternate reading frame (ARF) product from the ink4a locus that also codes for p16, has recently emerged as an additional player in the p53-Mdm2 system. p19ARF binds to and inhibits the actions of Mdm2 (P34,41). It also can bind to p53 (P40). Moreover, p19ARF is transcriptionally up-regulated by E2F1:DP1 (P42). This link between p53 and E2F1 may be crucial to the control of S-phase and apoptosis.

DNA Repair

The map includes three phases of nucleotide excision repair (NER). The first phase, lesion recognition and local opening of the DNA helix, is carried out by a molecular assembly, which includes the XPC:HR23B heterodimer (N1,2), XPA (N3), and TFIIH (N13). This phase opens the DNA helix in the vicinity of the lesion and allows access to other DNA repair proteins. If the DNA is opened by another process, such as transcription, XPC is dispensable, and repair can begin with the second phase.

The second phase, excision of a short DNA strand segment containing the lesion, is carried out by an assembly of the XPG and XPF:excision repair cross-complementing 1 (ERCC1) endonucleases (N6,8,9), together with XPA, RPA, TFIIH, and PCNA (which can bind XPG) (R9). This assembly appears to be held together in part through RPA, which binds to single-stranded DNA (ssDNA) regions in the vicinity of lesion (N10), and at the same time may be able to bind XPG and XPF:ERCC1 (N7), as well as XPA (N4). In going from the recognition to the excision phase, the molecular assembly rearranges as XPC:HR23B is replaced by XPG (N13) (Wakasugi and Sancar, 1998).

In the third phase, gap filling, x-ray repair cross-complementing gene/protein 1 (XRCC1) appears to function as a platform for the assembly of DNA polymerase β (DPase β) (N20), DNA ligase III (N19), and PARP (N18). This assembly is held together, in part, via breast cancer protein 1 C-terminal module (BRCT) modules in the constituent proteins (Masson *et al.*, 1998). The binding of PARP by XRCC1 may function to block the further action of PARP during this phase at a repair site. The assembly of XRCC1 with DPase β and DNA ligase III may also function in the single-nucleotide replacement pathway of base excision repair (Cappelli *et al.*, 1997).

Through its binding to DNA single-stranded regions, RPA may also recruit Rad52 and Rad51 to sites of DNA damage (S14,15). Rad51 also binds to ssDNA and, together with RPA, may function in recombinational repair (N11).

Rad51 may also provide links to a network of mutually interacting components via its binding to c-Abl (N12). c-Abl may bind ATM (A1), DNA-PK (B7), pRb (E18), and p53 (A4), although it is not fully determined which of these interactions can occur simultaneously and which are mutually exclusive. Rad51, ATM, and DNA-PK bind to a Src homology 3 (SH3) domain in the c-Abl N-terminal region, whereas pRb and p53 may bind to the c-Abl C-terminal region. c-Abl may regulate Rad51 function by phosphorylating Rad51 on Tyr54, thereby abrogating the direct binding of Rad51 to ssDNA (N12).

Another set of interactions is implicated in the processing of DNA double-strand breaks. DNA double-strand ends are recognized and bound by the Ku70:Ku80 heterodimer (Ku) (B1,2), which can recruit DNA-PK to the site (B3), thereby activating the kinase (B4). DNA-PK, however, can also bind to RPA (S16). RPA is a heterotrimer (S1,2) that binds ssDNA regions (S3). DNA-PK can thus be recruited to ssDNA regions formed transiently at replication forks. DNA-PK may then be available for interaction with a double-stranded DNA (dsDNA) end, which could appear in the vicinity as a consequence of replication fork encounters with open topoisomerase I DNA complexes trapped by drugs such as camptothecin (Shao *et al.*, 1999). It is noteworthy that DNA-PK does not always require Ku for activation, because it can be activated by tethering to DNA via other molecules, such as chromatin constituents of the HMG family (B10).

A further capability of RPA could arise from its ability to bind p53 (S6) and from the abrogation of this binding by phosphorylation of the RPA2 subunit by DNA-PK (S11), ATM (S12), or CycA:Cdk2 (S10).

We thus begin to see some of the intricate mechanism of the DNA repair machinery. This example shows how a molecular interaction map can represent DNA-targeted processes and the transitions between multimolecular assemblies.

DISCUSSION

Biological functions must eventually be understood as arising from molecular interactions. It is therefore not surprising that the molecular interaction information so far accumulated forms a highly complex network whose functional behavior may be difficult to comprehend. To provide a foundation for eventual understanding of function, this information must be organized in a manner that allows integrated behavior to be discerned. Molecular interaction maps such as those described here could contribute to this goal. The primary objective here was to suggest how complex molecular interaction networks can be usefully displayed. The map was constructed from evidence relating to molecular interactions with the view that the interaction patterns would suggest biological functions. The complexity of the map, however, demands that great care be taken in the formulation of specific functional hypotheses, which may have to be investigated with the aid of computer simulations (Kohn, 1998).

The molecular constituents were tentatively grouped into subsystems, demarcated on the map by dashed boxes. Some of the numerous connections between the boxes can be arranged in tracts, perhaps analogous to nerve tracts in the brain or communication buses in computers. A task for the future is to find an objective way to make these groupings, or indeed to determine to what extent nature has designed subsystems within the control network.

The map includes 26 individual p53 modification or binding interactions for which evidence of functionality has been presented. Considering the number of modifications and binding combinations that are possible a priori, the number of different possible states of p53 could be so large as to raise the question of whether, at any given time in a cell, any two p53 molecules would likely be in the same state. It seems plausible, however, that certain combinations of states would be strongly favored under particular circumstances.

p53 appears to be a central focus for the concurrence of signals from many pathways, presumably serving as an integrated information processor. It might, for example, function to test the validity of signaling patterns and, depending on the outcome of the test, to initiate cell cycle arrest or apoptosis. The test however must first be activated by allowing p53 (which normally is rapidly degraded and thus nearly absent) to accumulate in response to inputs involving Mdm2 and/or p19ARF. It appears to be a logic unit that monitors the state of the cell based on the pattern of a large number of inputs.

The molecular interaction map helps one discern possible multiprotein assemblies, alternative arrangements of which may operate under different circumstances in a cell or under particular states of cell differentiation. A recent example and possible paradigm is the large multiprotein binder and acetyl transferase p300, which may function as a platform for the assembly of high-order complexes (Grossman *et al.*, 1998). Multiprotein interactions may be competitive, cooperative, or independent of each other, possibly depending on the modification states of the component molecules. Moreover, susceptibility to modification may depend on the multimolecular configuration. A given protein may have more than one binding site for a second protein: p300, for example, has two separated sites for binding of p53 (Grossman *et al.*, 1998). Multimolecular complexes thus may have alternative, functionally different configurations, and covalent modifications could induce configurational switches. The details of these interdependencies may be critically important, but only fragmentary information is as yet available.

Promoter regulation can be very complicated, because transcription factors can have positive or negative effects, depending on their environments and mutual interactions. Genes controlled in part by the same transcription factors sometimes exhibit different regulation patterns. Moreover, the interactions among the different transcription factors are only beginning to be elucidated. It was therefore not feasible, in the present diagrams, to show the full regulation pattern of each individual gene. The regulation of the E2F-dependent S-phase genes and of the p53-dependent genes highlight the difficulties. The major known regulatory actions on these genes are shown (Figure 6, A and B), and some of the details regarding effects on individual genes are mentioned in the annotation list.

A diagram convention such as that described here will be needed for the representation of functional models. These "heuristic" diagrams do not fully define the contingencies among the interactions. For simulation of models, however, "explicit" or fully defined diagrams are needed. Suitable explicit diagrams can be prepared using a subset of the present symbols (Kohn, 1998) or using the conventions of electronic circuitry (McAdams and Shapiro, 1995).

This exercise has suggested how a large body of molecular interaction data can be organized in a map with associated annotations. Many of the known interactions relating to cell cycle control and DNA repair events in the nucleus were included in the map. Some important areas remain to be added: in particular, the control of nuclear-cytoplasmic shuttling of key regulatory molecules and the signaling pathways from growth factor receptors on the cell surface.

The molecular interaction map will require frequent updating as new information accrues. For this purpose, we

plan to put updated versions the map on the Internet (<http://discover.nci.nih.gov>)

APPENDIX: MOLECULAR INTERACTION MAP ANNOTATIONS

ATM and c-Abl

A1.

ATM is present in a complex that includes c-Abl and Rad51 (Yuan *et al.*, 1998).

A2.

ATM phosphorylates c-Abl on Ser465 and thereby activates the kinase (Baskaran *et al.*, 1997b).

A3.

Phosphorylation by ATM activates c-Abl (Baskaran *et al.*, 1997b). c-Abl tyrosine kinase activity is stimulated in response to ionizing radiation (IR), 1- β -D-arabinofuransylcytosine, camptothecin, or etoposide (Yuan *et al.*, 1996, 1998).

A4.

c-Abl binds to p53 in response to 1- β -D-arabinofuransylcytosine or methylmethanesulfonate (Yuan *et al.*, 1996); this interaction does not require c-Abl kinase activity. Moreover, c-Abl kinase activity does not require p53. Binding of c-Abl to p53 inhibits the Mdm2-mediated degradation of p53 (Sionov *et al.*, 1999).

A5.

c-Abl phosphorylates tyrosines in the C-terminal domain (CTD) of RNA polymerase II (RPase II; $K_m = 0.5 \mu\text{M}$) (Baskaran *et al.*, 1993, 1997a); the c-Abl SH2 domain is a specificity determinant for this reaction (Duyster *et al.*, 1995).

A6.

The c-Abl C-terminal region can bind the Crk SH3 domain; this interaction may link c-Abl function to the state of the cell surface with respect to integrins and focal adhesions (Gotoh and Broxmeyer, 1997).

A7.

c-Abl binds and tyrosine phosphorylates paxillin in an adhesion-dependent manner (Lewis and Schwartz, 1998).

A8.

c-Abl tyrosine kinase activity is blocked by pRb, which binds to the c-Abl kinase domain (Welch and Wang, 1995).

A9.

Phosphorylation of pRb disrupts the c-Abl:pRb complex and releases active c-Abl (Welch and Wang, 1995).

DNA Strand Break Processing

B1.

Ku is a tight heterodimer consisting of Ku70 and Ku80/86.

B2.

Ku loads onto dsDNA ends and can diffuse along the DNA in an energy-independent manner (deVries *et al.*, 1989). Ku can localize internally on dsDNA as well as at dsDNA ends (Yaneva *et al.*, 1997). Ku binds to DNA single-strand breaks (ssbs) (Blier *et al.*, 1993) and has helicase activity, but ssb-bound Ku does not activate DNA-PK (Smider *et al.*, 1998). Ku can also bind to hairpin-ended DNA without activating DNA-PK (Smider *et al.*, 1998).

B3.

Ku binds to the C-terminal region of DNA-PK (amino acids 3002–3850) near the protein kinase domain (Jin *et al.*, 1997). DNA-PK can bind weakly and transiently to dsDNA ends without Ku (Lieber *et al.*, 1997; Yaneva *et al.*, 1997; West *et al.*, 1998). In the presence of Ku, however, the binding is stronger and more stable. DNA-PK and Ku localize adjacent to each other at dsDNA ends. DNA-PK does not bind detectably to Ku in the absence of DNA. There is approximately five times more Ku than DNA-PK in mammalian cells.

B4.

The kinase activity of DNA-PK is stimulated by binding to dsDNA ends; however, the stimulation is greater in the complex with Ku (Yaneva *et al.*, 1997; West *et al.*, 1998).

B5.

DNA-PK phosphorylates itself, thereby blocking its interaction with Ku:DNA complex and inhibiting its kinase activity; it also phosphorylates Ku70 > Ku80, but without effect (Chan and Lees-Miller, 1996).

B6.

Autophosphorylated DNA-PK dissociates from Ku:DNA (Chan and Lees-Miller, 1996).

B7.

The SH3 domain of c-Abl binds to the C-terminal region of DNA-PK (amino acids 3414–3850) and may compete with Ku for binding to the same region (Jin *et al.*, 1997; Kharbanda *et al.*, 1997). c-Abl does not bind directly to Ku; however, IR induces the association of Ku with c-Abl:DNA-PK complex (Kharbanda *et al.*, 1997).

B8.

c-Abl phosphorylates DNA-PK in the C-terminal region (amino acids 3414–3850) (Jin *et al.*, 1997). c-Abl-dependent phosphorylation of DNA-PK is stimulated by IR (Kharbanda *et al.*, 1997). c-Abl does not phosphorylate Ku (Kharbanda *et al.*, 1997).

B9.

Phosphorylation of DNA-PK by c-Abl dissociates DNA-PK from Ku:DNA (Jin *et al.*, 1997; Kharbanda *et al.*, 1997).

B10.

HMG1 or 2 competes with Ku for binding to DNA-PK and stimulates DNA-dependent kinase activity in vitro in the absence of Ku (Yumoto *et al.*, 1998).

Cyclin-Cdk Box**C1.**

The Cyclin D1 promoter is activated by E2F4, but it is repressed by E2F1 via pRb (Watanabe *et al.*, 1998). In pRb-deficient cells, E2F1 stimulates this promoter. An Sp1 site close to the E2F element also participates in the regulation. (Overexpression of pRb can increase the expression of Cyclin D1 by an unknown mechanism [Watanabe *et al.*, 1998].)

C2.

The Cyclin E and A genes (but not the Cyclin D gene) are strongly activated by E2F1 (DeGregori *et al.*, 1995; Shan *et al.*, 1996). Further details about cyclin E promoter regulation have recently been reported (Le Cam *et al.*, 1999), as follows. In addition to a constitutively occupied E2F1-Sp1 site immediately upstream of the cyclin E transcription start region, there is downstream a cell cycle-regulated site (termed CERM) that may function as a cyclin E-repressor module. The CERM contains a variant E2F-recognition element, and binds a complex (termed CERC) consisting of E2F4, DP1, and either p130 or p107, as well as an unidentified necessary component.

C3.

Cdk4 and Cdk6 bind exclusively to D-type cyclins.

C4.

Cdk2 binds to cyclins E and A.

C5.

Cdk1 (Cdc2) binds cyclins A and B.

C6.

Cdk1 and 2 bind the small protein Cks1 (Jackman and Pines, 1997). Cks1 binds at the C-terminal region of Cdk, which is distinct from the region that binds cyclins. Cks1 may be involved in the dephosphorylation of Cdk Tyr15.

C7.

Low concentrations of p21Cip1, p27Kip1, or p57Kip2 promote the binding of Cyclin D to Cdk4 (LaBaer *et al.*, 1997), although high concentrations are inhibitory.

C8.

p16ink4a competes with Cyclin D1 for binding to Cdk4 (Hang *et al.*, 1998).

C9.

Cyclin D1 has a short half-life (<30 min), regardless of whether free or Cdk4-bound. Rapid degradation of Cyclin D1 requires phosphorylation at threonine-286 (kinase unknown, but not Cdk2 or Cdk4); degradation is by way of the ubiquitin-proteasome pathway (Diehl *et al.*, 1997).

C10.

D-type cyclins can bind the myb-like protein DMP1 (Hirai and Sherr, 1996). The binding does not require Cdk4/6 (Inoue and Sherr, 1998).

C11.

DMP1 binds consensus sequences CCCG(G/T)ATGT and activates transcription (Hirai and Sherr, 1996).

C11a.

DNA binding is inhibited when DMP1 is bound to cyclin D (Inoue and Sherr, 1998). Cyclin D binds at the DNA-binding domain of DMP1 immediately adjacent to the myb repeats (Inoue and Sherr, 1998).

C11b.

DMP1 activates transcription from the p19ARF promoter and induces cell cycle arrest and p21Cip1 accumulation in a p19ARF- and p53-dependent manner (Inoue *et al.*, 1999). Another DMP1-regulated gene is CD13/aminopeptidase N, which is activated cooperatively by DMP1 and c-Myb; its activation by DMP1 is inhibited by cyclin D independent of Cdk4/6.

C12.

When phosphorylated by Cyclin D-dependent kinases, DMP1 activates transcription (Hirai and Sherr, 1996).

C13.

Cyclin E has a half-life of ~30 min. It is degraded by way of the ubiquitin-proteasome pathway subsequent to phosphorylation (possibly autophosphorylation) at threonine-380 (Clurman *et al.*, 1996; Won and Reed, 1996). However, it can be stabilized by binding to Cdk2.

C14.

CycH:Cdk7 (also known as Cdk-activating kinase [CAK]) phosphorylates a site on the T-loop of Cdks (Thr161 in human Cdk1, Thr160 in Cdk2, Thr172 in Cdk4, and Thr170 in Cdk7 itself) and thereby causes the loop to be displaced to allow access to the catalytic site (Morgan, 1995). CAK readily phosphorylates Cdk2 monomer, which, however, remains inactive (Fisher and Morgan, 1994). This phosphorylation is required for the formation of stable CycA:Cdk1 dimer but not for the formation of other Cyclin:Cdk dimers (Ducommun *et al.*, 1991; Desai *et al.*, 1995). CAK is localized to the nucleus (Tassan *et al.*, 1994).

C15.

Cyclin B binds only to Cdk1. Cyclin B1 is retained in the cytoplasm (by means of a cytoplasmic retention sequence) until the time of mitotic prophase when it is abruptly transported into the nucleus (Pines and Hunter, 1994). Cyclin B1 is localized to microtubules and centrosome, whereas Cyclin B2 localizes to the Golgi complex (Jackman *et al.*, 1995).

C16.

Cdk1 is phosphorylated at Tyr15 by Wee1 and at Thr14 by Myt1 (Liu *et al.*, 1997, and references therein). Phosphorylations of Cdks are facilitated by Cyclin binding and stabilize the Cyclin:Cdk complex (Jackman and Pines, 1997). Human Myt1 phosphorylates and inactivates Cdk1 associated with cyclins A and B but does not phosphorylate Cdk2 or Cdk4 complexes (Booher *et al.*, 1997) (unlike wee1, which can phosphorylate both Cdk1 and Cdk2). Myt1 is membrane bound to endoplasmic reticulum and Golgi complex. Phos-

phorylation of Thr14 and Tyr15 occurs when the cycB:Cdk1 complex assembles in the cytoplasm (Pines and Hunter, 1994). Wee1, however, is in the nucleus; hence another kinase may be operating in the cytoplasm (Matsuura and Wang, 1996). Thr14 phosphorylation precedes Tyr15 phosphorylation (Liu *et al.*, 1997).

C17.

Phosphorylation of Thr14 or Tyr15 in Cdk1 reduces kinase activity 10-fold; phosphorylation of both sites reduces activity 100-fold (Liu *et al.*, 1997).

C18.

Dephosphorylation of Cdk1 Thr14 and Tyr15 sites is carried out by Cdc25C, which must itself be activated by phosphorylation in its N-terminal domain (Jackman and Pines, 1997). Cdc25C activity is high in mitosis during mitosis and low during interphase.

C19.

Cdk2 is regulated by phosphorylation at Tyr15, but there is much less phosphorylation at Thr14 (Booher *et al.*, 1997, and references therein).

C20.

Cdk4 may be inhibited by tyrosine phosphorylation (Terada *et al.*, 1995) but cannot be phosphorylated at the position corresponding to Thr14, because there is an Ala here rather than Thr.

C21.

p27Kip1 can be phosphorylated by Cyclin E- or Cyclin A-dependent kinases and thereby may be targeted for degradation (Sheaff *et al.*, 1997).

C22.

p21Cip1-induced inhibition of Cyclin:Cdk complexes requires the binding of more than one p21Cip1 molecule (Zhang *et al.*, 1994).

C23.

Cyclin A:Cdk2, in normal human fibroblasts, exists in complex with p21Cip1 bound to PCNA (Zhang *et al.*, 1993).

C24.

Raf1 can bind and activate Cdc25A (Galaktionov *et al.*, 1995; Weinberg, 1995), perhaps by phosphorylation.

C24a.

Raf1 is activated by Ras in a complex manner involving phosphorylations, as well as positive and negative effects of 14-3-3 interactions (Roy *et al.*, 1998; Thorson *et al.*, 1998; Tzivion *et al.*, 1998).

C25.

Cyclin A:Cdk2 is bound to p45Skp2 in complex with p19Skp1 in many transformed cells (Zhang *et al.*, 1995; Bai *et al.*, 1996).

C26.

p19Skp1 binds to an F-box motif in p45Skp2 (Bai *et al.*, 1996).

C27.

p45Skp2 inhibits Cdk2 kinase activity and blocks phosphorylation of Cdk2 by Wee1 or CAK (Yam *et al.*, 1999). Similarly to p21Cip1, two molecules of p45Skp2 seem to be required to inhibit Cdk2. Binding to p45Skp2 is mutually exclusive with p21cip1.

C28.

p45Skp2 (Ser76) can be phosphorylated by Cyclin A:Cdk2 (Yam *et al.*, 1999).

C29.

p19Skp1, through its F-box motif, may link Cyclin A to the ubiquitin-proteasome protein degradation device (Bai *et al.*, 1996).

C30.

Gadd45 binds to Cdk1 and inhibits Cdk1 activity, probably by displacing Cyclin B1 (Zhan *et al.*, 1999). Gadd45 may in this way contribute to the G2 delay response to some types of stress.

C31.

Cyclin D:Cdk4 phosphorylates pRb at a subset of sites, P^(D), but this does not suffice to abrogate the inhibition of E2F.

C32.

Cyclin E:Cdk2 phosphorylates pRb at additional sites, P^(E), after the Cyclin D:Cdk4-specific sites have been phosphorylated. (Cyclin E, in addition to acting on pRb, has actions that can induce S phase independent of pRb [Lukas *et al.*, 1997; Lundberg and Weinberg, 1998].)

C33.

Hyperphosphorylated pRb, resulting from combined phosphorylation by Cyclin D:Cdk4 and Cyclin E:Cdk2, abrogates the binding of pRb to E2F heterodimers (Lundberg and Weinberg, 1998).

C34.

p21Cip1 binds Gadd45 (Kearsey *et al.*, 1995).

C35.

Cdc25A may be transcriptionally activated by c-Myc; the Myc:Max heterodimer binds to elements in the Cdc25A gene and activates its transcription (Galaktionov *et al.*, 1996).

C36.

Cdc25C is activated by hyperphosphorylation of the N-terminal domain (Gabrielli *et al.*, 1997), which can be phosphorylated by Cyclin B:Cdk1 (Hoffman *et al.*, 1993).

C37.

The Cdc25C N-terminal domain can also be phosphorylated by Plk1 (Hamanaka *et al.*, 1994). During mitosis, Plk1,

by way of its polo boxes, localizes progressively to the centromeres, spindle poles, centrosomes, and spindle midzone or midbody (Glover *et al.*, 1998; Lee *et al.*, 1998c).

C38.

Chk1 binds and phosphorylates Cdc25A–C in vitro (Sanchez *et al.*, 1997). Cdc25C becomes phosphorylated at Ser216.

C39.

Cdc25C Ser216 also binds and is phosphorylated by C-TAK1 (Peng *et al.*, 1998). Cdc25C Ser216 is phosphorylated throughout interphase but not in mitosis (Peng *et al.*, 1997).

C40.

Ser216-phosphorylated Cdc25C is recognized and bound by 14-3-3 protein family members (Peng *et al.*, 1997, 1998). Ser216 phosphorylation and 14-3-3 binding probably sequester Cdc25C and thus prevent it from interacting with Cdk1 in vivo (Peng *et al.*, 1997). 14-3-3 σ is localized to the cytoplasm and may be the means by which Cdc25C is sequestered outside of the nucleus (Hermeking *et al.*, 1997).

C41.

Cyclin B is degraded late in mitosis through the ubiquitin-protein ligase (E3) activity of the anaphase-promoting complex, which is probably activated by phosphorylation by Plk1 (Glover *et al.*, 1998).

C42.

CycB:Cdk1 phosphorylates and inactivates the promoter selectivity factor SL1 (Heix *et al.*, 1998). This explains in part the silencing of rRNA synthesis during mitosis.

C43.

p16 associates with TFIIH and RNA polymerase II (RPase II) CTD and inhibits the phosphorylation of the CTD by TFIIH (Serizawa, 1998).

E2F-pRb Box

E1.

DP1 or 2 forms stable heterodimers with E2F1–6. (Interactions of E2F complexes have recently been reviewed [Dyson, 1998; Helin, 1998].)

E2.

Unphosphorylated (or hypophosphorylated) pRb can bind to DP complexes of E2F1–3 and to a lesser degree E2F4. Binding of pRb family members is mediated by a short, highly conserved domain in the C-terminal region of E2F proteins. The E2F-binding site is in the C-terminal region of pRb.

E3.

E2F4:DP can bind to p107 or p130 or, to a lesser extent, pRb.

E4.

E2F5:DP binds only to p130.

E5.

E2F1–5 complexes with DP1 or 2 can bind to E2 promoter elements, although there may be differences in preferences for variations of the E2 consensus sequence.

E6.

DP complexes of E2F1–5 can stimulate promoters containing E2 elements via a potent transactivation domain in the C-terminal region of the E2F component.

E7.

E2F-DP dimers, complexed with pRb, p107, or p130, can bind and inhibit E2 promoter elements (Dyson, 1998; Mayol and Grana, 1998). In quiescent cells, the predominant complexes contain E2F4 and p130.

E8.

E2F6:DP complexes bind to a variation of the E2 consensus sequence, possibly competing with other E2F complexes (Cartwright *et al.*, 1998).

E9.

In contrast to other E2F species, E2F6:DP directly represses transcription. E2F6 lacks a transactivation domain; it has instead a repression domain in its C-terminal region (Gaubatz *et al.*, 1998). E2F6:DP represses a subset of E2F-responsive genes (Cartwright *et al.*, 1998). E2F6:DP does not bind to pRb, p107, or p130 (Trimarchi *et al.*, 1998).

E10.

E2F4 is protected against proteasomal degradation when associated with p130 (Hateboer *et al.*, 1996).

E11.

Upon stimulation of quiescent cells by growth factors, p130 becomes hyperphosphorylated and incapable of binding E2F (the responsible kinase is unidentified) (Mayol and Grana, 1998). Hyperphosphorylated p130 is unstable. Upon growth factor stimulation of quiescent cells, p130 declines late in G1 and is replaced by p107, which is absent in quiescent cells (Mayol and Grana, 1998; Nevins, 1998).

E12.

p130 may associate with HBP1, a transcription factor involved in cell cycle exit during differentiation (Tevosian *et al.*, 1997).

E13.

HDAC1 binds to pocket protein family members pRb, p107, and p130 and is thereby recruited to E2F complexes on promoters (Ferreira *et al.*, 1998). The binding is via an IXCXE motif in HDAC1, which can bind to the C-terminal region of p130 (Stiegler *et al.*, 1998) and presumably to the LXCXE site on the B-box of pRb (Lee *et al.*, 1998b).

E14.

The interaction with HDAC1 enhances transcriptional repression by pocket proteins (Brehm *et al.*, 1998; Ferreira *et al.*, 1998; Luo *et al.*, 1998).

E15.

E2F-regulated genes include many that are involved in cell cycle progression and control. Individual genes are differently regulated. Dihydrofolate reductase (*DHFR*) is activated via the E2F transactivation domain, whereas *B-myb*, *Cyclin E*, *E2F-1*, *E2F-2*, and *Cdc2* are regulated via the repression domain of pRb family proteins (Dyson, 1998).

E16.

pRb binds and activates C/EBP (Chen *et al.*, 1996a,b). The binding of C/EBP to its DNA recognition elements is enhanced.

E17.

Hypophosphorylated pRb binds c-Jun, JunD, and JunB (Nead *et al.*, 1998). This enhances the binding of the Jun family members to c-Fos and stimulates transcriptional activation by the Fos:Jun complexes. A region (amino acids 612–657) in the B-pocket of pRb and a region in the C-pocket can independently bind c-Jun. The binding site in c-Jun is in the leucine zipper region.

E18.

pRb binds c-Abl via the pRb C-pocket (residues 768–785 and 825–840). pRb can bind c-Abl and E2F simultaneously. (Welch and Wang, 1995; Whitaker *et al.*, 1998). The c-Abl-binding C-pocket and the E2F-binding CTD of pRb are distinct from each other.

E19.

pRb binds Mdm2 via the pRb C-terminal 44 residues (Xiao *et al.*, 1995; Tan and Wang, 1998). These C-terminal residues are not required for the growth-suppressive effect of pRb.

E20.

CycA:Cdk2 binds to E2F1–3 at a site near the N-terminal region, as a consequence of which both the E2F and DP component are phosphorylated. Phosphorylation of either impairs the binding between the E2F and DP monomers (Xu *et al.*, 1994; Krek *et al.*, 1995; Dynlacht *et al.*, 1997).

E21.

The p107 promoter contains E2F recognition elements and can be repressed by pRb or p107 (Zhu *et al.*, 1995).

E22.

Raf1 can bind pRb and p130, which are not thereby dissociated from E2F complexes, although promoter inhibition is reversed (Wang *et al.*, 1998). There was no detectable binding to p107. Binding to pRb is mediated by the N-terminal 28 amino acids of Raf1. The kinase activity of Raf1 was required to reverse the pRb-mediated promoter repression (Wang *et al.*, 1998), but the phosphorylation sites on pRb remain to be described and therefore are not indicated in the diagram.

E23.

Sp1 cognate elements are found in the promoter regions of several S-phase genes that also contain E2F elements, including *DHFR*, *c-myc*, thymidine kinase, *cyclin E*, and *E2F1* (Datta *et al.*, 1995). Binding of Sp1 and E2F to the chromatin-

organized thymidine kinase promoter was cooperative (Karlseder *et al.*, 1996).

E24.

Sp1 and E2F1 bind to each other (Karlseder *et al.*, 1996; Lin *et al.*, 1996a; Watanabe *et al.*, 1998). Sp1 binds to the N-terminal region of E2F1; this region is also present in E2F2 and E2F3 but not in E2F4 and E2F5; accordingly, Sp1 can bind E2F1–3 but not E2F4 or 5 (Karlseder *et al.*, 1996). The Sp1-binding region of E2F1 may overlap the cyclin A-binding region. It is, however, separated from the transactivation and pRb-binding regions, which are near the E2F1 C terminus. E2F1 binding requires the C-terminal region of Sp1 where Zn fingers are located (Karlseder *et al.*, 1996; Lin *et al.*, 1996a). (Sp1 may function as a higher-order complex; see Karlseder *et al.*, 1996.) The E2F1-binding region of Sp1 is phosphorylated by an Sp1-associated kinase when quiescent cells are induced to proliferate (Black *et al.*, 1999).

E25.

Sp1 and E2F binding sites are both essential for activation of the murine thymidine kinase promoter (Karlseder *et al.*, 1996). The promoter was activated when the Sp1 and E2F sites were separated by 6 or 10 bp but not when they were separated by 20 bp. The *DHFR* promoter was strongly activated by Sp1 alone but hardly at all by E2F1 alone (cotransfection in insect cells) (Lin *et al.*, 1996a). E2F1, however, enhanced the activating ability of Sp1, even in the absence of a functional E2F binding site on the promoter.

E26.

Sp1 binds to p107 (within the first 385 amino acids of p107), which is separate from the p107 pocket region that binds E2F4 (Datta *et al.*, 1995).

E27.

p107 inhibits Sp1-dependent transcription. Binding of p107 to Sp1 seems to inhibit the binding of Sp1 to DNA (Datta *et al.*, 1995).

Chromatin and Acetylase Box**H1.**

HDAC1 removes acetyl groups from histones, thereby making nucleosomes compact and inhibitory to transcription (i.e., HDAC1 removes acetyl groups that inhibit the inhibitory effect of compact nucleosomes on transcription; thus there is an odd number [3] of negative effects, which resolves to a net negative effect).

H2.

Gadd45 binds to core histones in chromatin or nucleosomes whose structure has been loosened by acetylation or UV light (UV) radiation (Carrier *et al.*, 1999).

H3.

p300 and CBP have intrinsic histone acetyl transferase activity (Ogryzko *et al.*, 1996).

H4.

p300 binds PCAF (Grossman *et al.*, 1998, and references therein).

H5.

p300 binds to the transactivation domain of E2F1 (Lee *et al.*, 1998a). E2F1 and p53 may be reciprocally regulated by their mutual dependence on coactivation by limiting amounts of p300 (Lee *et al.*, 1998a).

H6.

The p300 C-terminal region can bind Cyclin E:Cdk2 (Perkins *et al.*, 1997).

H7.

p300, via its Cys/His-rich region C/H3, associates with RPAse II via the intermediacy of RNA helicase A, which can bind both RPAse II and the C/H3 domain (Nakajima *et al.*, 1997).

Myc Box**M1.**

c-Myc and pRb compete for binding to AP2 (Batsche *et al.*, 1998).

M2.

AP2 and Max compete for binding to c-Myc (Batsche *et al.*, 1998). AP2 and Myc associate in vivo via their CTDs (Gaubatz *et al.*, 1995).

M3.

The E-cadherin promoter is regulated via AP2 recognition elements (Hennig *et al.*, 1995, 1996; Batsche *et al.*, 1998).

M4.

c-Myc and pRb enhance transcription from the E-cadherin promoter in an AP2-dependent manner in epithelial cells (mechanism unknown) (Batsche *et al.*, 1998). Activation by pRb and c-Myc is not additive, suggesting that they act on the same site, thereby perhaps blocking the binding of an unidentified inhibitor. No c-Myc recognition element is required for activation of the E-cadherin promoter by c-Myc. Max blocks transcriptional activation from the E-cadherin promoter by c-Myc, presumably because it blocks the binding between c-Myc and AP2.

DNA Repair**N1.**

XPC forms a tight complex with HR23B, a homologue of yeast Rad23. HR23B is present in large excess over XPC (Sugasawa *et al.*, 1997).

N2.

The XPC:HR23B complex may be the primary recognizer of a variety of DNA lesions and the initiator of the NER of nontranscribed DNA regions (Sugasawa *et al.*, 1998). XPC is required to open the DNA to allow access of other repair factors, such as XPA and RPA, to the vicinity of the lesion

(Evans *et al.*, 1997a,b). XPC is not required for transcription-coupled repair, perhaps because the lesion-containing DNA region is opened by the encounter with the transcription machinery (Mu and Sancar, 1997; Sugasawa *et al.*, 1998).

N3.

XPC is necessary to promote the stable binding of XPA to UV-damaged DNA (Li *et al.*, 1998). XPA binds to DNA and preferentially at sites of bulky damage produced, for example, by UV, cisplatin, or N-AAF. However, the association constant of XPA for UV-irradiated DNA is only severalfold above that for unirradiated DNA, suggesting that other factors (such as XPC) may be required for effective lesion recognition (Jones and Wood, 1993; Sugasawa *et al.*, 1998).

N4.

RPA binds directly to XPA via the C-terminal region of RPA2 (He *et al.*, 1995; Stigger *et al.*, 1998).

N5.

XPA binds ERCC1 (residues 93–120) (Li *et al.*, 1994) ($K_d = 2.5 \times 10^{-7}$ M; Saijo *et al.*, 1996).

N6.

The XPF C-terminal region (residues 814–905) binds to the C-terminal region of ERCC1 (residues 224–297) (de Laat *et al.*, 1998b).

N7.

RPA binds XPG and ERCC1:XPF, the NER endonucleases (He *et al.*, 1995; Matsunaga *et al.*, 1996; for review, see Wold, 1997).

N8,9.

The ERCC1:XPF heterodimer incises the damaged DNA strand 15–24 nucleotides to the 5' side of the lesion (Mu *et al.*, 1995). XPG and ERCC1:XPF cut on the 3' and 5' sides of the lesion, respectively (de Laat *et al.*, 1998a, and references therein). RPA binding enhances the activity of XPG and ERCC1:XPF (Matsunaga *et al.*, 1996). RPA is required for the nucleotide excision process (Moggs *et al.*, 1996; Mu *et al.*, 1996).

N10.

RPA binds single-strand regions at locally unwound intermediates in NER (Evans *et al.*, 1997). The RPA:XPA complex binds cooperatively to DNA damage sites (He *et al.*, 1995).

N11.

Rad51-coated ssDNA, together with Rad52 and RPA, stimulate strand exchange in homologous recombination (Bauman and West, 1997; New *et al.*, 1998) (also see S14).

N12.

Rad51 binds to c-Abl directly (Yuan *et al.*, 1998). c-Abl phosphorylates Rad51 Tyr54, as a consequence of which the binding of Rad51 to ssDNA is inhibited (Yuan *et al.*, 1998). IR induces c-Abl-dependent phosphorylation of Rad51 (Yuan *et al.*, 1998).

N13.

XPG binds to and further stabilizes the open DNA complex with XPA, RPA, and TFIIH; at the same time, XPC:HR23B dissociates from the DNA complex (Wakasugi and Sancar, 1998). XPG and XPC:HR23B appear to be mutually exclusive in binding to complexes containing TFIIH. XPC and XPA, as well as ATP hydrolysis, are required for the stable binding of TFIIH to DNA lesions (Li *et al.*, 1998). RPA (at least the p34 subunit) is present together with XPA and TFIIH in the open complex (Li *et al.*, 1998).

N14.

PARP binds to dsDNA termini, as well as ssbs.

N15.

DNA-PK is ADP ribosylated by PARP *in vitro*, and the protein kinase activity (including p53 and RPA substrates) is thereby stimulated (Ruscetti *et al.*, 1998). (PARP is phosphorylated by DNA-PK [Ruscetti *et al.*, 1998], but the consequences of this are unknown; therefore, this reaction is not included in the chart.)

N16.

PARP is phosphorylated at a serine and a threonine site by PKC α and β *in vitro*, as a consequence of which PARP binding to DNA is weakened, and PARP activity is reduced (Bauer *et al.*, 1992).

N17.

PARP binds and stimulates DPase α (Simbulan *et al.*, 1993). PARP was found to be physically associated with the catalytic subunit of the DPase α -primase tetramer during the S or G2 phase of the cell cycle but not in G1 (Dantzer *et al.*, 1998). PARP has been found to be associated with an active multiprotein replication complex consisting of ~40 proteins, 15 of which were found to be poly(ADP) ribosylated, including DPase α , DPase δ , topoisomerase I, and PCNA (Simbulan-Rosenthal *et al.*, 1998).

N18.

PARP binds XRCC1 by way of their respective BRCT (BRCA1 C-terminus) modules (Masson *et al.*, 1998). This interaction inhibits the catalytic activity of PARP. The PARP:XRCC1 complex is not dissociated by 1 M NaCl.

N19.

XRCC1 binds DNA ligase III by way of BRCT modules (Masson *et al.*, 1998). XRCC1 has two BRCT modules: one at residues 314–402 binds PARP; the other is at the C terminus (residues 538–633) and binds DNA ligase III at a C-terminal BRCT domain (residues 841–922) (Masson *et al.*, 1998).

N20.

XRCC1 binds DPase β (Masson *et al.*, 1998). XRCC1 can bind simultaneously to PARP, DNA ligase III, and DPase β to form a multiprotein assembly that may function in the short patch pathway of base–excision repair (Masson *et al.*, 1998).

N21.

BRCA1 and BRCA2 colocalize with Rad51 in discrete nuclear foci (Scully *et al.*, 1997a,b; Chen *et al.*, 1998). BRCA1 residues 758–1064 bind Rad51 *in vitro*. In response to DNA damage or drug-induced DNA synthesis inhibition, BRCA1 and Rad51 relocate to PCNA-containing replication structures; this is accompanied by phosphorylation of BRCA1 (dependent neither on ATM nor on DNA-PK) (Scully *et al.*, 1997a,b; Chen *et al.*, 1998).

p53 Box**P1.**

Casein kinase 1 δ and ϵ have been reported to phosphorylate serines 4, 6, and 9 *in vivo* (Knippschild *et al.*, 1997). p53 up-regulates casein kinase 1 δ (but not 1 ϵ) (Knippschild *et al.*, 1997). Phosphates at N-terminal amino acids 1–15 turn over rapidly (McKendrick and Meek, unpublished data; cited by Meek, 1998); it is not known which protein phosphatase is responsible.

P2.

ATM phosphorylates Ser15 (but not Ser37) *in vitro* (Banin *et al.*, 1998; Canman *et al.*, 1998). IR or UV induces Ser15 phosphorylation (Siliciano *et al.*, 1997). In ATM-defective cells, the Ser15 phosphorylation response to IR is delayed, but this response to UV is normal. IR-induced posttranslational modification may stimulate p53 transcriptional activity (Siliciano *et al.*, 1997). ATM communicates radiation-induced DNA damage to p53 (Kastan *et al.*, 1992). ATM phosphorylates p53 at Ser15 in response to IR (Shieh *et al.*, 1997; Siliciano *et al.*, 1997). ATM-dependent phosphorylation of Ser15 was observed in response to double-strand breaks produced by microinjected *Hae*III and was required for p53 stabilization (Nakagawa *et al.*, 1999). The ATM-related protein can phosphorylate p53 at both Ser15 and Ser37 in response to IR or UV (Tibbetts *et al.*, 1999).

P3.

Casein kinase 1 δ phosphorylates Thr18, but only after Ser15 has been phosphorylated (Appella, personal communication).

P4.

Thr18 phosphate turns over rapidly through the action of an unidentified phosphatase.

P5.

Phosphorylation of Ser18 prevents stable binding of p53 to Mdm2 (Appella, personal communication). The ability of Mdm2 to inhibit p53-dependent transactivation was reported to be impaired by phosphorylation of Ser15 and Ser37 (Shieh *et al.*, 1997), but this may occur indirectly via stimulated phosphorylation of Ser18 (Appella, personal communication). Recent evidence implicates phosphorylation of Ser20 in the inhibition of Mdm2 binding and consequent p53 degradation (Shieh *et al.*, 1999; Unger *et al.*, 1999). These phosphorylation sites are within the region required for Mdm2 binding. Phosphorylation of Ser20 is rapid after IR but delayed after UV (Shieh *et al.*, 1999).

P6.

DNA-PK phosphorylates Ser15 and Ser37 (Shieh *et al.*, 1997). Phosphorylation of p53 by DNA-PK is heavily dependent on the presence of DNA double-strand ends (Shieh *et al.*, 1997).

P7.

c-Jun N-terminal kinase 1 (stress-activated protein kinase 1a) phosphorylates only serine 33 (Milne *et al.*, 1995; Milne and Meek, unpublished data; cited by Meek, 1998). The stress-activated protein kinase-like kinase that phosphorylates murine serine-34 (corresponding to human serine-33) is activated after treatment of cells with UV (Milne *et al.*, 1995). (The kinase is inactivated by a phosphatase.)

P8.

T73 and T83 were phosphorylated by activated recombinant p42-MAP kinase but not by inactive MAP kinase or by the activating protein MAP kinase (Milne *et al.*, 1994). Evidence suggested that this may occur in vivo.

P9.

CycA:Cdk2 and CycB:Cdc2 (but not CycE:Cdk2 or CycD:Cdk4) phosphorylate serine-315 in vitro (Wang and Prives, 1995).

P10.

PKC phosphorylates Ser378 (Takenaka *et al.*, 1995). However, the phosphorylation of p53 by PKC in vitro may not correlate with the ability of PKC to enhance transcriptional activation by p53 (Youmell *et al.*, 1998).

P11.

The Cdk7:CycH:p36 (CAK) component of TFIIH phosphorylates p53 in vitro, enhancing its sequence-specific DNA binding activity (Lu *et al.*, 1997). The phosphorylation was within C-terminal region 311–393, which includes serines-371, -376, -378, and -392 as potential sites. The relevant site may be Ser392. This kinase can also phosphorylate Ser33, at least in vitro (Ko *et al.*, 1997).

P12.

Ser376 becomes dephosphorylated in response to IR, probably by activation of an unidentified phosphatase by ATM (Waterman *et al.*, 1998).

P13.

Phosphorylation of Ser378 creates a recognition site for the binding of 14-3-3 (Waterman *et al.*, 1998). It is not known which 14-3-3 family members are involved.

P14.

14-3-3 probably binds as a homodimer.

P15.

Phosphorylation of Ser376 blocks the Ser378-binding site for 14-3-3.

P16.

Binding of 14-3-3 at the Ser378 site enhances the sequence-specific DNA binding of p53 (Waterman *et al.*, 1998), probably by blocking the nonspecific DNA binding.

P17.

Ser392 phosphorylation increases 10-fold the association constant for tetramer formation by the p53 tetramerization domain (Sakaguchi *et al.*, 1997). Ser315 phosphorylation largely reversed the effect Ser392 phosphorylation. Ser392 phosphorylation activates p53 for specific DNA binding and transcription (Hupp and Lane, 1994; Hao *et al.*, 1997), probably because of stabilization of p53 tetramers. Murine Ser389 (corresponding to human Ser392) is phosphorylated in response to UV but not IR or etoposide (Kapoor and Lozano, 1998; Lu *et al.*, 1998).

P18.

At the normally low p53 concentration in cells, p53 should be largely monomeric (Sakaguchi *et al.*, 1997). At elevated p53 concentrations and/or increased association constant attributable to Ser392 phosphorylation, a sharp transition to the tetramer may be expected.

P19.

Casein kinase 2 (CK2) phosphorylates murine Ser389 (human Ser392) (Kapoor and Lozano, 1998).

P20.

PCAF acetylates p53 Lys320 (Sakaguchi *et al.*, 1998).

P21.

p300 acetylates p53 Lys382 (Sakaguchi *et al.*, 1998).

P22.

Acetylation of Lys320 or Lys382 enhances sequence-specific DNA binding (Gu and Roeder, 1997; Sakaguchi *et al.*, 1998).

P23.

Acetylation of p53 by PCAF is strongly inhibited when Ser378 is phosphorylated (Sakaguchi and Appella, personal communication).

P24.

p300 coactivates p53-activated promoters, including p21, Bax, and Mdm2 (Avantaggiati *et al.*, 1997; Lill *et al.*, 1997; Thomas and White, 1998). It is not known whether histone acetylation contributes to this coactivation. p300, and not its close relative CBP, is involved in this action (Yuan *et al.*, 1999).

P25.

The p300 C-terminal region binds the transactivation domain in the N-terminal region of p53 (Grossman *et al.*, 1998; Lee *et al.*, 1998a; Sakaguchi *et al.*, 1998). This interaction is involved in the coactivation of p53 by p300, perhaps via acetylation of p53 and/or histones.

P26.

A p53 N-terminal region (distinct from that which binds Mdm2) binds to DP1 and competes with E2F1 for binding to DP1 (Sorensen *et al.*, 1996).

P27.

p53 CTD binds the TFIIH-associated DNA helicases XPD and XPB (ERCC2 and 3), as well as CSB which is involved in strand-specific DNA repair (Wang *et al.*, 1995; Lu *et al.*, 1997).

P28.

p53 N-terminal peptide (residues 17–27) binds as amphipathic α -helix in a hydrophobic cleft in the Mdm2 N-terminal region (residues 17–125) (Kussie *et al.*, 1996).

P29.

Mdm2 inhibits p53-mediated transactivation (Momand *et al.*, 1992). The binding of Mdm2 to the p53 transactivation domain blocks its interaction with TAF_{II}70 and TAF_{II}31 (discussed by Freedman and Levine, 1998). Thus, in addition to inhibiting gene activation by p53, Mdm2 can suppress the basal transcription of p53-dependent genes.

P30.

p53 is short-lived ($t_{1/2}$ = 5–40 min, depending on cell type) (Reihnsaus *et al.*, 1990). p53 degradation occurs by way of ubiquitination and proteasome (Maki *et al.*, 1996).

P31.

Mdm2 stimulates p53 degradation (Bottger *et al.*, 1997; Haupt *et al.*, 1997; Kubbatat *et al.*, 1997; Midgley and Lane, 1997), apparently by functioning as an E3 ubiquitin ligase that specifically recognizes p53 (Honda *et al.*, 1997; Fuchs *et al.*, 1998). Mdm2 contains a nuclear export signal, as well as a nuclear import signal; the ability of Mdm2 to shuttle between nucleus and cytoplasm is required for Mdm2-mediated p53 degradation (Freedman and Levine, 1998; Roth *et al.*, 1998; Freedman and Levine, 1999). Mdm2 transports p53 from nucleus to cytoplasm where p53 is degraded (Tao and Levine, 1999).

P32.

The Mdm2-induced ubiquitination of p53 is reduced when p53 is phosphorylated at the DNA-PK sites (Ser15 and 37) (Honda and Yasuda, 1999).

P33.

The C/H1 domain near the N terminus of p300 binds both Mdm2 and the core domain of p53 (Grossman *et al.*, 1998). p300 binds independently to both p53 and Mdm2. Much of the Mdm2 in the cell may exist in stable complex with p300. The site of Mdm2 binding is in the vicinity of the p300 C/H1 domain (residues 342–414) (Grossman *et al.*, 1998). The C/H1 region of p300 may function as a platform that positions these molecules to facilitate the Mdm2-dependent ubiquitination p53 (Grossman *et al.*, 1998) (also see P31).

P34.

The p19ARF exon 1 β -encoded N-terminal domain binds to the Mdm2 C-terminal region (Zhang *et al.*, 1998b). p19ARF binds to Mdm2 in a region that overlaps the p300-binding domain, suggesting that p19ARF may compete with p300 for Mdm2 binding (Pomerantz *et al.*, 1998).

P35.

The Mdm2 N terminus (residues 1–220) associates with E2F1:DP1 and/or pRb and enhances the transcriptional activity of E2F1:DP1 (Martin *et al.*, 1995; Xiao *et al.*, 1995). (There is a sequence resemblance between p53 and E2F1 that may correspond to a common binding site.)

P36.

TBP binds to an acidic domain in central Mdm2 (residues 221–272) (Leveillard and Wasylyk, 1997; Thut *et al.*, 1997).

P37.

The C-terminal region of Mdm2 (residues 432–489) binds to an HMG-like region of TAF_{II}250 and activates the Cyclin A promoter (Leveillard and Wasylyk, 1997).

P38.

Caspase-3 cleaves Mdm2 at a specific site between the central domain and CTD (Chen *et al.*, 1997; Erhardt *et al.*, 1997).

P39.

The Mdm2 gene can be activated via a p53-responsive intronic promoter (Juven *et al.*, 1993; Zauberman *et al.*, 1995). This may be part of a negative feedback control of p53 actions (Wu *et al.*, 1993). Mdm2 appears to be expressed in all tissues at all times (Montes *et al.*, 1996).

P40.

p19ARF can bind directly to p53 as well as to Mdm2 (Kamijo *et al.*, 1998). Ternary complexes can form with Mdm2 as the bridging molecule.

P41.

p19ARF inhibits the Mdm2-dependent ubiquitination and degradation of p53 (Pomerantz *et al.*, 1998; Stott *et al.*, 1998; Honda and Yasuda, 1999). p19ARF and Mdm2 colocalize in nucleoli (Pomerantz *et al.*, 1998). Ectopic expression of p19ARF extended the half-life of p53 from 15 to ~75 min (Kamijo *et al.*, 1998). Zhang *et al.* (1998b) reported that p19ARF promotes Mdm2 degradation in HeLa cells. Coexpression of p19ARF reduced the half-life of ectopically expressed Mdm2 from 90 to 30 min. In murine embryonic fibroblasts, however, p19ARF caused endogenous Mdm2 to accumulate (Kamijo *et al.*, 1998; Sherr, 1998), and this action appears to be independent of p53. One way or another, p19ARF antagonizes Mdm2 function by a mechanism that does not require increased Mdm2 degradation (Sherr, 1998).

P42.

E2F1 directly activates the p19ARF gene promoter and induces the expression of p19ARF protein (Bates *et al.*, 1998). A potential E2F1 binding site (GCGGGAAA) was noted upstream from the p19ARF initiation codon. This may be how E2F1 can stimulate p53-mediated apoptosis (discussed by Prives, 1998).

P43.

p53 binds to a consensus site in the p21 gene in response to IR (Chin *et al.*, 1997; el-Deiry, 1998).

P44.

p53 binds to the Gadd45 gene at a site in the third intron where there is a single p53-binding consensus sequence (p53 does not bind to the Gadd45 promoter region) (Kastan *et al.*, 1992). p53 is necessary for induction of the Gadd45 gene in response to IR (but not for induction in response to methylmethanesulfonate, UV, or starvation medium). IR induced

binding of p53 to its consensus site in the Gadd45 gene (Chin *et al.*, 1997). However, p53 can participate in transcriptional induction of the GADD45 promoter in the absence of direct DNA binding (Zhan *et al.*, 1998).

P45.

p53 induces the 14-3-3 σ gene; there is a p53-binding element 1.8 kb upstream from the transcription start (Hermeking *et al.*, 1997; el-Deiry, 1998). 14-3-3 σ is strongly induced by IR, and ectopic expression of 14-3-3 σ causes a G2 arrest response similar to that produced by p53, suggesting that the 14-3-3 σ family member is involved in the suppression of Cdc25C (Hermeking *et al.*, 1997).

P46.

p53 binds PARP in vitro and in vivo (Vaziri *et al.*, 1997); the p53 N-terminal region (residues 1-72) is mainly responsible. p53 can bind a DNA consensus sequence and PARP simultaneously. p53 can be ADP-ribosylated in vitro, and this p53 modification prevents binding to its consensus sequence (Wesierska *et al.*, 1996).

P47.

p53 binds BRCA1; this binding enhances the transcriptional activity of p53 (Ouchi *et al.*, 1998; Zhang *et al.*, 1998a). The interacting regions map to BRCA1 residues 224-400 (where there is a BRCT domain) and to the p53 C-terminal region.

P48.

c-Fos is transcriptionally activated by p53 through a p53-recognition element located in the first intron of the c-fos gene (Elkeles *et al.*, 1999).

P49.

DNA-PK can phosphorylate Mdm2 at Ser17 in vitro and thereby inhibit the binding of Mdm2 to p53 (Mayo *et al.*, 1997).

P50.

A functional p53 site resides within intron 2 of KARP1 (Myung *et al.*, 1998). KARP1 mRNA is induced by IR in a p53- and ATM-dependent manner, and KARP1 protein accumulates after IR. KARP1 is transcribed from the same locus as Ku80. Transcription of Ku80, however, is not p53 dependent and not induced by IR. (Ku80 transcription begins within exon 3 of KARP1, the p53 site being approximately equidistant between the KARP1 and Ku80 transcription start sites.) Constitutive expression of Ku80 is normally greater than that of KARP1 by 1-2 orders of magnitude, but p53 greatly increases KARP1 protein levels without much change in Ku80 (Mysung *et al.*, 1997; Myung *et al.*, 1998).

P51.

Bax appears to be transcriptionally activated by p53, although this seems to occur only in apoptosis-competent cells (Selvakumaran *et al.*, 1994a,b; Zhan *et al.*, 1994a).

P52.

HMG1 binds p53 and enhances p53 binding to DNA, as well as p53-mediated transcriptional activation (Jayaraman *et al.*, 1998). This may be due in part to HMG1-induced DNA

bending and assembly of a nucleoprotein structure containing p53.

Replication Box

R1.

p19Skp1 is a subunit of CBF3, which binds centromere DNA (Connelly and Hieter, 1996).

R2.

DPase α -primase may be regulated by phosphorylation of its p68 subunit by Cyclin A:Cdk2, which reduces and Cyclin E:Cdk2 which increases its DNA initiation activity (Voitenleitner *et al.*, 1999).

R3.

PCNA (as a trimer) forms a topological ring around dsDNA and functions as a sliding clamp (Tinker *et al.*, 1994).

R4.

Clamp-loader RF-C loads PCNA onto the DNA (Kelman, 1997). RF-C interacts with the C-terminal side of PCNA (Mossi *et al.*, 1997).

R5.

PCNA binds to Pol δ and enhances the processivity of DNA replication (for review, see Kelman, 1997).

R6.

p21Cip1 (CTD) binds PCNA and inhibits replication, perhaps by dissociating PCNA from Pol δ (for review, see Kelman, 1997). Assembly of PCNA around DNA by RF-C is not substantially inhibited. The K_d of the p21:PCNA complex is 2.5 nM (Gibbs *et al.*, 1997).

R7.

PCNA binds to flap endonuclease 1 (FEN-1; DNase IV) (Kelman, 1997). The PCNA:FEN-1 complex processes branched DNA intermediates (Wu *et al.*, 1996).

R8.

PCNA binds DNA ligase I; the complex may function to join Okazaki fragments during DNA replication (Levin *et al.*, 1997).

R9.

PCNA binds repair endonuclease XPG (Gary *et al.*, 1997).

R10.

PCNA binds Gadd45 (Smith *et al.*, 1994; Chen *et al.*, 1995). This interaction enhances NER. PCNA binds Gadd45 and p21Cip1 competitively.

R11.

Cyclin D binds PCNA directly and inhibits PCNA-dependent replication (Matsuoka *et al.*, 1994; Pagano *et al.*, 1994).

R12.

p53 binds and transiently transactivates the PCNA promoter (when p53 reaches high levels, transactivation seems to stop) (Shivakumar *et al.*, 1995; Morris *et al.*, 1996; Xu and Morris, 1999).

ssDNA Processing

S1,2.

RPA is a tight heterotrimer of p70RPA1, p34RPA2, and p14RPA3 subunits. The RPA2:RPA3 dimer binds to the C-

terminal region of RPA1, whereas neither RPA2 nor RPA3 forms soluble dimers with RPA1 (for review, see Wold, 1997). Direct binding has been demonstrated between RPA1 and RPA2 and between RPA2 and RPA3 but not between RPA1 and RPA3 (Kim *et al.*, 1996; Lin *et al.*, 1996b). RPA functionality probably occurs only with intact RPA trimer; binding to RPA2:RPA3 may be required for proper folding of RPA1 (Henricksen *et al.*, 1994; Stigger *et al.*, 1994).

S3.

RPA binds tightly to ssDNA ($K_a =$ of 10^9 – 10^{11} M^{-1}) and can also bind to sites of DNA damage where short ssDNA segments may appear because of disturbed base pairing (for review, see Wold, 1997). Binding may occur to bubbles as small as four nucleotides. RPA binds preferentially to pyrimidine-rich sequences. The binding tends to be cooperative. Binding to ssDNA makes RPA a better substrate for phosphorylation (Blackwell *et al.*, 1996; Gomes *et al.*, 1996). The strongest and probably initial site of ssDNA binding is to the RPA1 central domain (Kim *et al.*, 1996); subsequent binding to weaker sites on RPA2 and RPA3 extends the binding from an eight-nucleotide to a longer region (Bochkarev *et al.*, 1997).

S4.

RPA binds DPase α via the N-terminal half of RPA1 and stimulates the activity of DPase α /primase and DPase δ (Braun *et al.*, 1997; Wold, 1997). This is at least in part due to stabilization of ssDNA segments during replication and is helped by the binding of RPA to DPase α . Although ssDNA binding is primarily via RPA1, stimulation of replication requires the intact RPA trimer.

S5.

The C-terminal region of RPA2 also binds the DNA base-excision enzyme U-glycosylase (Nagelhus *et al.*, 1997).

S6.

The N-terminal region of RPA1 binds to the N-terminal region of p53 (amino acids 41–73) (Wold, 1997).

S7.

The binding of RPA to p53 inhibits the binding of RPA to ssDNA (Dutta *et al.*, 1993) and the binding of p53 to its promoter sites (Miller *et al.*, 1997).

S8.

Human RPA2 can be phosphorylated at several Ser/Thr sites within the N-terminal 33 amino acids (Henricksen *et al.*, 1996). At least five phosphorylation states have been resolved (Zernik-Kobak *et al.*, 1997). In unirradiated cells, RPA2 is phosphorylated primarily at Ser23 and Ser29 (Dutta and Stillman, 1992; Pan and Hurwitz, 1993). DNA damage by UV, IR, camptothecin, or etoposide induces phosphorylation of additional sites (Zernik-Kobak *et al.*, 1997; Shao *et al.*, 1999). These hyperphosphorylations are attributable to DNA-PK (Shao *et al.*, 1999) and independently to ATM (Gately *et al.*, 1998).

S9.

Cyclin A (but not Cyclin E) binds to RPA2 (Gibbs *et al.*, 1996).

S10.

Cyclin A:Cdk2 and Cyclin A:Cdc2 phosphorylate RPA2; the major site for Cdc2 has been mapped to Ser29 (Niu *et al.*, 1997).

S11.

DNA-PK phosphorylates RPA2 at Thr21, followed by Ser33 (Niu *et al.*, 1997).

S12.

ATM (or an ATM-associated kinase other than DNA-PK or ATM-related protein) hyperphosphorylates RPA2 on serine and threonine residues in response to DNA damage; the reaction is dependent on both ssDNA and linear dsDNA (Gately *et al.*, 1998).

S13.

DNA damage-induced hyperphosphorylation of RPA2 prevents the binding of RPA to p53 (Abramova *et al.*, 1997).

S14.

Human RPA binds Rad52; this interaction may be essential for homologous recombination (Park *et al.*, 1996). The CTD of RPA2 may be involved in an interaction with Rad52 (Stigger *et al.*, 1998).

S15.

RPA also binds Rad51, but more weakly than Rad52 (Golub *et al.*, 1998); the binding involves the C-terminal region of RPA1 (residues 168–327). In addition, Rad51 and 52 may bind to each other (Milne and Weaver, 1993), suggesting that RPA, Rad51, and Rad52 function in concert in homologous recombination (also see N8). RPA colocalizes with Rad51 in nuclear foci induced by DNA damage (Golub *et al.*, 1998).

S16.

DNA-PK can bind to RPA1 (Shao *et al.*, 1999).

S17.

Phosphorylation of RPA2 by DNA-PK impairs the binding of RPA to DNA-PK (Shao *et al.*, 1999).

S18.

KARP1 stimulates DNA-PK activity and may function in complex with DNA-PK and Ku (Mysung *et al.*, 1997; Myung *et al.*, 1998) (also see P50).

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