One billion years of p53/p63/p73 evolution

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The report in this issue of PNAS by Joerger et al. (1) solves the structure of the p73 oligomerization domain that produces a tetrameric protein and compares this structure with p63 (closely related) and p53 (more distantly related). This provides new information about the comparative evolution of these three (p53, p63, and p73) proteins and more.

The human genome contains three genes encoding the transcription factors p53, p63, and p73, which are closely related paralogs with some similar and some diverse functions (Fig. 1*A*). p53 acts in somatic cells in response to stresses (such as DNA damage) that reduce the fidelity of DNA replication and cell division, by initiating apoptosis, senescence or cell cycle arrest, providing a tumor suppressor function (2). Humans heterozygous for a p53 mutation develop a variety of cancers at early ages (3). p63 is one of the major transcription factors required for the development of epithelial cell layers. Humans carrying a mutation in one allele of this gene can have cleft palate, skeletal abnormalities, and skin pathologies (EEC syndrome), but they do not develop cancers at high rates (4, 5). p73 is involved in the development of the central nervous system and the immune system. It also responds to a subset of stress signals observed with p53, initiating apoptosis, and in this sense p73 can back up p53 in response to DNA damage (6). Studies with knock-out mice have indicated that p53, p63, and p73 can also play a role in germ-line surveillance of fidelity (7–9), and p63 and p73 may be involved in aspects of tumor suppression. p53 also is required for efficient implantation of embryos into the uterus in mice (10) and humans (11). It is the germ-line surveillance and implantation functions that result in strong evolutionary selection pressures on this family of genes (12). The p53, p63, and p73 proteins are structurally related. They all have an amino-terminal transcriptional *trans*-activation domain linked to a DNA-binding domain, which is followed by an oligomerization domain (a tetramer in vertebrates) and a carboxyl-terminal regulatory region (Fig. 1*A*). p63 and p73 have an extra SAM (sterile alpha motif) domain, which confers protein stability. The DNA-binding domains of p53, p63, and p73 have been conserved over long evolutionary time frames, with the three human paralogs sharing 55–87% homology, and the *Drosophila* gene (dmp53) having 25% homology, whereas the *C. elegans* gene (Cep-1) has 15% homology at the amino acid level (12). Both invertebrate and vertebrate proteins recognize and bind to the same DNA sequences that regulate some similar genes in these very diverse species, leading to apoptosis after a DNA-damaging event. dmp53 and Cep-1 play a central role in germ-line fidelity in response to a variety of stress signals (DNA damage, starvation, etc.).

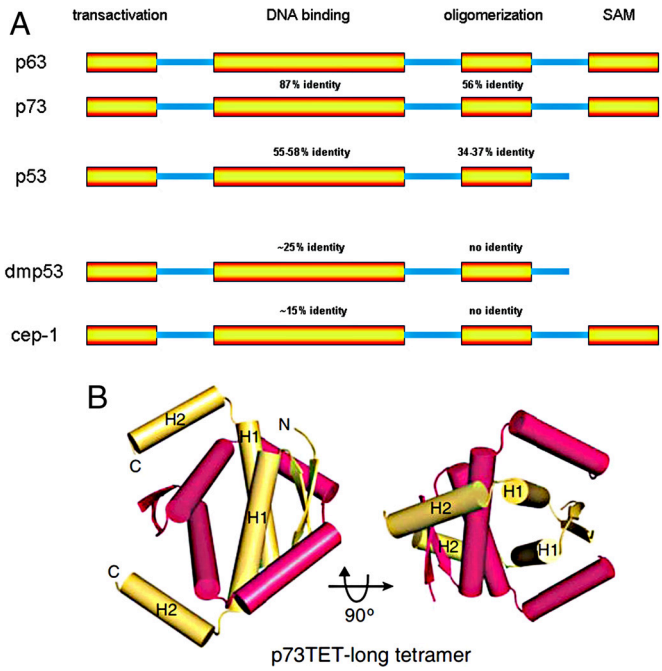

Fig. 1. The domain structure of the p53 family proteins. (*A*) The domain organization of human p53, p63, and p73 along with the p63/p73 ancestor proteins from *Drosophila melanogaster* and *Caenorhabditis elegans*. The percentage of identity of the amino acids and their position in the domain are given for both the DNA-binding domain and the oligomeric domain. (*B*) The structure of the oligomeric domain of p73 from ref. 1. The H-2 helix stabilizes this p73 tetramer and is absent from the p53 tetramer. p63 and p73 are more closely related to each other than to p53 based on the primary, secondary, tertiary, and quaternary structures of both the DNA-binding domains and the oligomerization domains.

Most invertebrates harbor a single p63/p73-like gene, with the earliest organism observed during evolution, the sea anemone, expressing this ancestor gene in its germ cells. When exposed to ultraviolet light, which happens when these organisms feed at the surface of the water, the p63/73 ancestor gene initiates apoptosis to protect the germ line from mistakes (12). This means that the structure and the functions of the p53 sister genes have been preserved for about one billion years. The first indication that this ancestor p63/p73 gene duplicated, separating into distinct entities, is in the cartilaginous fish (shark), where a new p53 gene is observed along with a single p63/p73 ancestor gene (12). By the appearance of bony fish (zebra fish) all three genes, p53, p63, and p73, are present and p53 has taken on its new functions ensuring the fidelity of somatic cell division, an adaptation of the p63/p73 ancestor genes' role in the germ line (12). Within the higher vertebrates, p63 and p73 have taken on new functions in development of tissues and organs, whereas p53 has become the guardian of the somatic genome and a tumor suppressor. With the advent of employing large numbers of stem cells and tissue regeneration as a strategy for an organism's growth, development, and maintenance, there is a greater need for stem cell surveillance to prevent cancers from arising. p53 evolved to fill this role. It is

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of some interest that p53 has recently been shown to regulate the efficiency of induced pluripotent stem cell production from differentiated cells (13–17) indicating a new possible role for p53 in enforcing the direction of developmental processes in a cell. There is an intimate relationship among p53, stem cell development, and epigenetic regulation of these processes, and it began to evolve in the fishes.

The structure of the oligomerization domain of p73 (Fig. 1*B*) gives us new information about the structural evolution of the three sister proteins and more (1). The p73 tetrameric domain, like the p53 oligomerization domain (18), is a dimer of a dimer composed of monomeric blocks containing a short β-strand followed by an α-helix (H-1 helix). What is distinct about the p73 and the p63 oligomerization domain, compared with p53, is the presence of an extra helical region (the H-2 helix) that stabilizes the overall architecture of the p73 (and presumably p63) tetramer. The H-2 helix acts as a clasp packing against the H-1 helix (Fig. 1*B*). In the absence of the H-2 helix the tetramer is much less stable, as measured by thermal denaturation in differential scanning calorimetry. These structural differences in the human oligomerization domains of p63 and p73 (56% identical at the amino acid level) separate them from a more distant p53 (34–37% identical to p63 and p73) as previously observed with the DNA-binding domains (p63/p73 are 87% identical whereas p53/p63-p73 are 55–58% identical). This agrees well with the detection of the first p53 gene duplicating from a common p63/p73 ancestor gene in the evolutionary studies of the genomes of fish (1, 12).

Humans heterozygous for a p53 mutation develop a variety of cancers at early ages.

Joerger et al. (1) go on to examine the exchange in vitro of p63 and p73 monomeric units in the oligomerization domains to form hybrid proteins. This exchange is slow (≈10 h at 37 °C) and does not occur with p53 monomers or dimers (18). For several reasons this is an important biological question that is not yet resolved. First, we would like to know whether the combinations of mixed heteromeric p63/p73 exist in vivo and have any functional significance. There are multiple isoforms, or splice forms, of both proteins that could produce a complex picture in development. Second, in cancers a mutant missense p53 protein can show a gain-of-function phenotype both in cell culture (19) and in vivo (20). One hypothesis that helps to explain this observation is that mutant p53 proteins form complexes with other transcription factors, such as p63 or p73, and either inactivate them or change their patterns of transcription, altering the properties of the cancer cell. Indeed, mutant p53/p73 complexes have been detected in cancer cells (21), and an altered chemotherapeutic response could be the result of an inactivation of p73 apoptotic functions (22). This is one of the phenotypes of mutant p53 in a cancer cell. The Joerger et al. article suggests that formation of this heterotetrameric mutant p53/p73 protein is not likely mediated by the oligomeric domain, but elsewhere in the two proteins. If we wish to develop drugs that break this complex and free p73 to act as apoptotic mediator of chemotherapy (21–23) it will be useful to know the locations in the proteins where they interact to form mixed tetramers. A common polymorphism in the domain of the p53 protein that connects the *trans*-activating domain with the DNA-binding domain (not in the oligomerization domain) can affect the mutant p53/p73 heterodimer formation (23) consistent with all of these observations and suggesting a new location for protein–protein interactions. Although this question of a mutant p53 gain of function and its possible mechanism remains unresolved, its importance for understanding both the poor outcomes in cancers associated with mutant p53 and possible new methods of treatment of cancers with mutant p53 makes this an important issue for future study.

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