showed that compatible partners mated effectively within a mammalian host during an experimental infection, but they were unable to demonstrate mating in vitro on agar plates. In contrast, Magee and Magee showed that mated progeny arose spontaneously, albeit more slowly, on selective agar plates (they did not investigate mating of *C. albicans* in animal hosts).

One explanation for the differing results could be that non-*MTL* genes on chromosome 5 might directly or indirectly affect mating. The *C. albicans MTL* locus is much larger than the *S. cerevisiae MAT* locus, and contains several additional genes that encode two phosphoinositol kinases, two oxy-sterol binding proteins, and two polyadenylate polymerases. Deletion of these genes along with the *MTL* genes seems to reduce mating efficiency (*2*). Chromosome 5 genes located outside the *MTL* locus (of which the Magee and Magee organisms had only one copy) may also affect mating frequency. Also, the different temperatures and the contrasting environmental conditions in vitro and in vivo may account for the observed differences in the efficiency and rate of mating of *C. albicans* between the two studies.

Does mating in *C. albicans* occur at any meaningful frequency in nature, and if so, does it generate a tetraploid intermediate (see the figure)? Aneuploid organisms may arise from time to time in the human host, but possibly not with sufficient frequency for compatible mating partners to meet. Hull *et al.* report that a tetraploid cell, generated by one of the induced mating sessions, underwent spontaneous random chromosome loss. Thus, a tetraploid cell might undergo chromosome loss or meiosis to restore the diploid condition. Alternatively, *C. albicans* might undergo meiosis naturally to generate true haploids; these haploids might exist only transiently and only under favorable conditions. Completion of the sexual reproductive cycle might depend on the rapid mating of such haploids during infection of a mammalian host, before the possible deleterious consequences of the entire genome becoming haploid take effect. There are many examples in nature of haploid gametes that are not independently viable and exist only as a transient stage of the life cycle.

It will be now important to examine the formal possibility that *C. albicans* not only mates, but also undergoes meiosis. Significantly, the *Candida* genome sequencing project has revealed homologs of many of the genes in *S. cerevisiae* that are required for meiosis (*14*). The discovery of a full sexual cycle in *C. albicans* will be important for understanding how this fungus has coevolved with its human host. It will also have a major impact on the future of molecular genetics in *C. albicans*. Our detailed knowledge of *S. cerevisiae* biology is based firmly on classical genetic methods that exploit the sexual cycle in this yeast.

These reports provide a salutary lesson to us all. As a direct result of genome sequencing, one of the firmest tenets of the biology of a highly studied microorganism is now being questioned. This is yet another striking example of what the study of genomes can teach us about basic biology.

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PERSPECTIVES: DEVELOPMENT

# p73—Guilt by Association?

**Richard S. Morrison and Yoshito Kinoshita**

Just because proteins have similar amino acid sequences does not mean that they do the same job. The rapidly expanding p53 family of transcription factors exemplifies this phenomenon. The founding member of this family, p53, activates the expression of target genes engaged in promoting growth arrest or cell death in response to genotoxic stress (DNA damage). Mutations in p53 are frequently found in human tumors, and so p53 is often called a tumor suppressor protein. Long considered an orphan, the only one of its kind, p53 has now been found to have two close relatives, p63 and p73. The amino acid identities of these two proteins are conserved within critical functional domains suggesting that, like p53, they may also be involved in the regulation of growth arrest and cell death. However, a wealth of new studies, including the report on p73 (*1*) by Pozniak *et al.* (page 304 of this issue), clearly demonstrate that despite being evolutionarily conserved, members of the p53 family have distinct, even antagonistic biological activities.

The diversity in biological activity among p53 family members stems from several sources including differences in mRNA processing. The *p53* gene generates a single species of mRNA with one open reading frame. In contrast, transcription of the *p63* and *p73* genes generates several alternatively spliced mRNA transcripts (*2*, *3*). Point mutations in the *p53* gene have long been known to alter the activity of the p53 protein (especially its DNA-binding capabilities). However, p53 studies provided no foreshadowing of the contribution of alternative mRNA splicing to the biological diversity of p63 and p73.

There is a *p73* mRNA variant, transcribed under the influence of an alternative promoter located in intron 3 of the *p73* gene, that has a truncated amino terminus (*3*). This truncated form of p73 lacks the transactivation domain, which is essential for activating the expression of p73- and some p53-responsive genes. Similarly, a truncated form of *p63* missing its amino terminus can prevent full-length p63 and p53 proteins from activating transcription of their target genes. Thus, p53 family members lacking a transactivation domain are dominant negative inhibitors of p53-dependent processes.

When overexpressed, full-length p73 shares with p53 the ability to activate common target genes, resulting in growth arrest or apoptosis (*4*–*6*). Despite the structural similarities between full-length p73 and p53 and their display of some common characteristics in culture assays, these proteins differ in several important respects. In contrast to mice that are missing p53, animals lacking all forms of p73 do not exhibit an increased propensity to form tumors (*3*). Moreover, p53-deficient mice develop normally, whereas p73-deficient animals have profound defects in brain development. Thus, p53 and p73 may be involved in different aspects of nervous system development.

The authors are in the Department of Neurological Surgery, University of Washington School of Medicine, Box 356470, Seattle, WA 98195–6470, USA. E-mail: yael@u.washington.edu

That p53 is an important regulator of apoptotic cell death in the nervous system is suggested by the finding that p53 expression is elevated in damaged neurons in acute models of injury, such as those for ischemia and epilepsy, and in brain tissue samples from patients with chronic neurodegenerative diseases (*7*). Furthermore, in the absence of p53, neurons survive a wide variety of acute toxic insults. The p53 tumor suppressor protein is also essential for the death of select neuronal populations during development. In p53-deficient mice and even in animals with only one *p53* allele, there is a reduction in the number of sympathetic neurons that die during development (*8*).

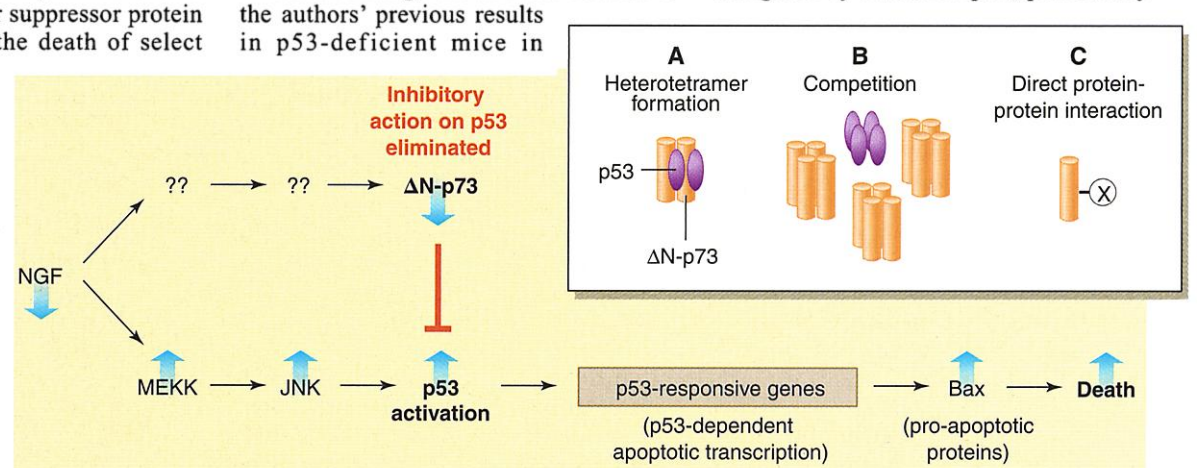
To determine what p73 does during mouse development, Pozniak and colleagues (*1*) first characterized the expression of *p73* mRNA variants in the neonatal mouse brain and in the sympathetic superior cervical ganglia. Surprisingly, a truncated p73 variant lacking an amino terminus is the predominant form in developing neurons in vivo and in cultured neonatal sympathetic neurons (*1*). When nerve growth factor (NGF), a neuronal survival factor, is withheld from cultured sympathetic neurons, these cells undergo p53-dependent cell death (*8*). Contrary to expectations, NGF withdrawal elicits a decline in truncated p73 in sympathetic neurons rather than an increase as would be predicted if, like p53, it is a pro-apoptotic protein. To verify that the decline in truncated p73 promoted the death of sympathetic neurons, Pozniak *et al.* used an adenovirus delivery system to maintain expression of the truncated form after NGF withdrawal. Sympathetic neurons infected with adenovirus encoding truncated p73 were rescued from cell death, whereas those infected with a control adenovirus were not (*1*). These results suggest that truncated p73 is actually an anti-apoptotic protein in neurons.

Next, Pozniak and co-workers analyzed sympathetic neurons in neonatal mice deficient in p73 to determine whether truncated p73 suppresses neuronal cell death in vivo. If truncated p73 is necessary for survival of sympathetic neurons, then the absence of this form would be expected to promote death of these cells during development. This is exactly what the authors found: The size of the two superior cervical ganglia and the number of neurons that each contained decreased in p73-deficient mice compared with normal littermates.

These findings are in direct contrast to the authors' previous results in p53-deficient mice in which sympathetic neurons are prevented from dying during normal development (*8*). One explanation for the new result could be that truncated p73 directly interacts with p53 to block its transcriptional and pro-apoptotic activities (see the figure). This hypothesis is supported by the demonstration that p53 binds to truncated p73. Furthermore, infection of sympathetic neurons with an adenovirus containing *p53* causes them to die (*1*, *9*), whereas coinfection with vectors encoding both p53 and truncated p73 nullifies this effect.

Collectively, the results of the Pozniak study demonstrate that truncated p73 is an essential anti-apoptotic protein in neurons. It will be interesting to determine whether this protein is up-regulated in neurons of the central nervous system in response to injury or disease. If it can be shown that truncated p73 blocks the death of neurons in response to various harmful insults, this protein may hold therapeutic promise for rescuing injured neurons. Clearly, the demonstration of truncated p73's anti-apoptotic activity has significant implications for other cell types because it is the predominant p73 form in most adult and embryonic tissues (*3*).

Truncated p73 may control p53-dependent processes by directly binding to p53. It will be important to determine the full range of p53-dependent activities that are abrogated by truncated p73, particularly because p53 may promote apoptosis through gene repression or by transcription-independent pathways (*10*, *11*) that depend on p53 binding to other proteins (*12*, *13*). Thus, we should not judge p73 by the company it keeps because it is clear that the various forms of p73 behave quite differently from other members of the p53 family.




**Live and let die.** During normal development, about half of all sympathetic neurons in the superior cervical ganglion die off. **(Top)** A limited supply of NGF maintains suboptimal levels of truncated p73, an anti-apoptotic protein. **(Bottom)** It also activates the stress-activated protein kinase (SAPK/JNK) signaling pathway, which up-regulates the pro-apoptotic protein, p53. This tumor suppressor protein stimulates the expression of other pro-apoptotic proteins such as bax. Expression of truncated p73 in response to the NGF survival signal could be maintained through activation of the Ras/phosphatidyl inositol 3-kinase/Akt and/or the Ras/MEKK/MAPK signaling pathways, which counteract the apoptotic pathway driven by MEKK/JNK/p53/bax (*14*). When NGF levels are limited (blue arrows), the JNK/p53/bax pathway is accentuated because truncated p73 levels decline, eliminating a principal block of p53 activity. Truncated p73 interacts with p53 and suppresses p53-mediated cell death in three possible ways: **(A)** by forming heteromeric tetramers with p53, preventing it from binding to the promoters of its target genes; **(B)** by competitive inhibition of p53 binding to these promoters; or **(C)** by direct protein-protein interactions with unidentified binding partners (X) that are required to produce an apoptotic signal.

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