Citation: Cell Death and Disease (2011) 2, e162; doi:10.1038/cddis.2011.45

www.nature.com/cddis

News and Commentary

NF-Y joins E2Fs, p53 and other stress transcription factors at the apoptosis table

R Gatta[1], D Dolfini[1] and R Mantovani*,[1]

Cell Death and Disease (2011) **2,** e162; doi:10.1038/cddis.2011.45; published online 26 May 2011

Most of the key effectors of apoptosis are constitutively expressed in cells and ready to operate; however, activation of diverse transcription factors (TFs), specific for noxious conditions, eventually leads to alteration of the transcriptome and expression of proapoptotic proteins. Several recent reports pointed at NF-Y as yet another TF having a – previously unsuspected – role in the process. The obvious question – pro or anti? – is more difficult to answer, as the current data delineate a regulatory tale, rather than a simplistic 'good *versus* bad guy' script.

NF-Y is a heterotrimer composed of two histone-fold domain (HFD)-subunits NF-YB/NF-YC and of NF-YA, which provides the capacity to bind avidly to the CCAAT sequence in transcriptional regulatory regions.[1] NF-YA is the limiting subunit of the complex, tightly regulated by post-translational modifications (PTMs) of lysines; acetylation by the coactivator KAT3 protects NF-YA from polyubiquitination-mediated degradation, prolonging an otherwise relative short half-life.[2] Gurtner *et al.*[3] tried to stably overexpress NF-YA in cells, an experiment bound to failure, including in our hands. Rather than giving up, they went on showing that cells undergo apoptosis, and most importantly, that this can be corrected by transfecting fibroblasts, genetically ablated of E2F1 or p53. Apparently, a rather modest increase of E2F1, whose expression is under NF-Y control, is believed to drive the process. So, too much NF-Y, or NF-YA in this case, is bad, but what about too little? Imbriano and co-workers[4] functionally ablated the three subunits by shRNA interference and found that NF-YA elimination leads to a remarkable apoptotic response. The suicide is preceded by a delay in S-phase progression, which unleashes p53 activation and a DNA-damage response. These data are in agreement with previous mouse genetic data obtained with KO technology; embryogenesis was stopped very early, with cells affected by severe defects in S-phase progression.[5] On the other hand, inactivation of NF-YB or NF-YC caused a different defect, namely a delay in G2/M exit,[4] which is quite surprising, considering that all subunits are required for DNA-binding. The difference might have to do with separate roles of NF-YA and the HFD dimer in DNA metabolism, related to specific groups of genes whose transcription might be differentially sensitive to depletion of the single subunits. What do we know about the genes regulated by NF-Y?

Thanks to ChIP-on-chip, profilings and bioinformatic analysis, the NF-Y regulome is relatively well understood,[1] by and large, pro-survival, pro-growth genes are found aplenty and the anti-apoptotic Bl-1, Bcl-xl and Bcl-2 are controlled directly.[6] Interestingly, there is a difference in the transcriptome upon NF-YA or NF-YB inactivations; in the latter, the most notable term in GO analysis is cell cycle, among which G2/M genes stand out,[4,6] in line with the notorious abundance of CCAAT boxes in G2/M promoters.[1] This is very well in keeping with the phenotypic effects of shRNA inactivation. Genes inhibited by NF-YA inactivation, instead, mostly belong to metabolism terms, which is not immediately translatable into a phenotype of S-phase progression impairment, nor activation of the DNA-damage response.[4] In general, it is far from apparent to discern an enrichment of proapoptotic terms among genes controlled by NF-Y. The expression of proapoptotic CCAAT-less Bax, PUMA, NOXA and BIK is increased upon NF-YB removal, suggesting an indirect effect, which is indeed essentially absent in $p53^{-/-}$ cells.[6] Collectively, these data would argue that NF-Y positively controls an antiapoptotic program and that the overexpression/inactivation effects are secondary to the activation of other TFs (Figure 1).

There are, however, important caveats to this interpretation. The first came from ChIP-on-chip assays showing that NF-Y is associated not only to active, but also to inactive loci, which are activated after NF-Y removal; interestingly, apoptosis was one of the few terms that popped up in GO analysis of this repressed subset.[7] The second comes from a recent report on transcriptional control of Bim, a strong proapototic gene, essential to mediate cell death of sympathetic neurons upon NGF withdrawal. Ham and co-workers[8] showed that increased Bim transcription is the result of cooperation between FOXO3 and NF-Y, to recruit the KAT3 coactivator.[9] Finally, findings of Emerson and co-workers[10] made the matter even more intriguing; by dissecting the p53-mediated activation of the proapoptotic Fas/APO1 gene promoter, they could not find a classic p53 responsive element, but stumbled on an NF-Y site, instead. In fact, the site is very degenerate and found in an unusual location, downstream of the transcriptional start site rather than in the typical −80 area. In essence, such deviant site(s) would go completely unnoticed in a TFBS inspection of promoters, even

[1]Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy
*Corresponding author: R Mantovani, Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy.
Tel: +39 025 031 5005; Fax: +39 025 031 5044; E-mail: mantor@unimi.it

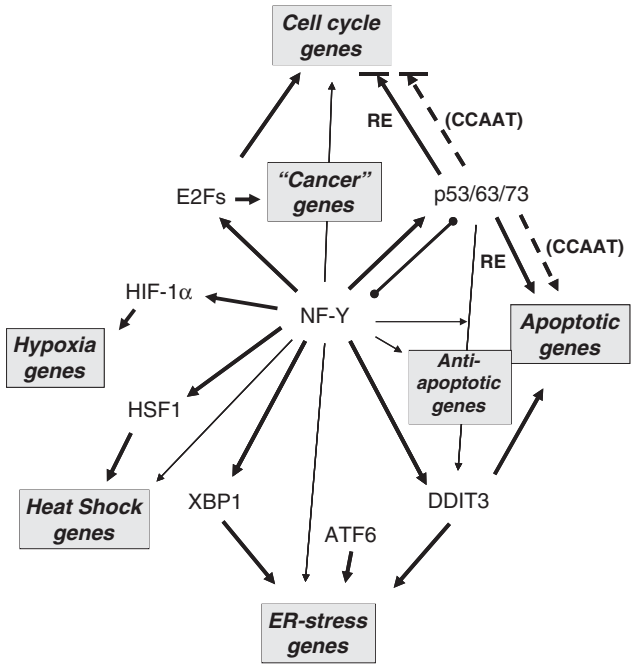

Figure 1 The arrows connect NF-Y to TFs involved in the different processes. Dotted line refers to indirect activation/repression through CCAAT boxes by p53/p63/p73. The line between NF-Y and p53/p63/p73 indicates a direct interaction between the TFs

with the upgraded NF-Y PSFM we recently introduced.[1] The obvious question is, therefore, how many deviant NF-Y sites in proapoptotic genes are being missed simply because of their unorthodox location and/or sequence. Note that previous examples of CCAAT degeneracy almost certainly points at the necessity to accommodate an overlapping site for another TF.[1] In summary, NF-Y proapoptotic primary target genes exist, and the cohort is likely larger and more genetically diversified than previously thought; connections with specific TFs, direct or allowing binding to adjacent sites, are probably equally crucial. Another important aspect of the Morachis *et al.*[10] paper is that activation of Fas/APO1 through NF-Y/p53 is temporarily delayed, but longer lasting with respect to p21, which functions on *bona fide* p53 REs. The activatory scenario is different from previous findings on the repressive role of p53 on cell-cycle promoters of the G2/M phase,[11] shared by p63 and p73,[12,13] but it does explain the data of NF-YA over-expressions,[2] reinstating the importance of the 'special' partnership between NF-Y and p53.[6]

Not much is known about the NF-Y-p53 combination of sites at the genomic level, but CCAAT and E2F elements are emerging in important pathways. First, bioinformatic analysis of all RefSeq CCAAT promoters for TFBS enrichments indicates E2F as very high in the ranking, particularly in cell cycle promoters.[1] Second, profilings of primary cells undergoing an *in vitro* transformation process by p53 and p16INK4A inactivation identified a G2/M genes signature, whose architecture relied upon NF-Y and E2F sites.[14] Third, *de novo* motifs discovery in promoters of large cohorts of genes overexpressed in different tumors led to the identification of a core of three TF sites: the NF-Y and E2F *duo* (Figure 1), and surprisingly, p53.[15] Clearly, precise ChIP-Seq inspection of the mutual locations is now required to further detail the interplay of NF-Y with specific E2F family members.

The proapoptotic table is crowded with additional stress-specific TFs, whose altered expression and/or function is notoriously associated with apoptosis. The promoters of CHOP and XBP1, masters of the ER-stress response, of HSFs (heat shock response) of HIF-1α (hypoxia) as well as of p53/p63, have a CCAAT in a 'perfect' location and are under transcriptional control of NF-Y[1](RM, unpublished, Figure 1). The ER-stress genetic program is a good example of a coordinated response, collectively activated by ATF4/ATF6, XBP1 and Gadd153/CHOP/DDIT3.[16–18] All these TFs function on a landscape predisposed by NF-Y binding[16] (references therein). Eventually, damaged cells are committed to cell death, which is mediated by the same TFs. ATF6 and XBP1 are mainly controlled at the post-transcriptional level, whereas CHOP/DDIT3 is activated transcriptionally in the initial phases of the response, and it is the key mediator of apoptosis.[18] A recent paper illustrates the strategic connections leading to a global response; cisplatin treatment of squamous cell carcinomas activates ATM-mediated p63 phosphorylation which, in turn, causes a proapoptotic response by activation of CHOP transcription via tethering of phospho-p63 on the CCAAT/NF-Y complex of the CHOP promoter.[19] Profiling and ChIP-on-chip analysis demonstrated a widespread presence of CCAAT boxes in activated genes, and the relative NF-Y binding, in promoters regulated by phospho-p63. Many promoters of the DNA-damage and ER-stress response are bound by NF-Y under 'basal', uninduced conditions,[16,20] and cooperation with pathway-specific TFs has been shown in many downstream targets.[1]

In summary, a better understanding of the role of NF-Y in apoptosis will come from a more detailed genomic analysis of NF-Y sites, positive and negative, and the matching with the regulomes of fellow TFs involved in apoptosis induction. A structural understanding of the interactions with p53 and members of the family, and possibly E2Fs, might explain why NF-YA inactivation is sensed as an immediate DNA-damage danger by the cells. Finally, an additional, untapped layer of complexity resides in the structural and PTM features of the HFD dimer; whether the pro- or antiapoptotic behavior of NF-Y is locally influenced by specific PTMs of the HFD subunits, still very much a black box at the moment, or NF-YA for that matter, is an appealing possibility.

Conflict of Interest

The authors declare no conflict of interest.

1. Dolfini D *et al. Cell Cycle* 2009; **8**: 4127–4137.
2. Manni I *et al. Mol Biol Cell* 2008; **19**: 5203–5213.
3. Gurtner A *et al. Cancer Res* 2010; **70**: 9711–9720.
4. Benatti P *et al. Nucleic Acids Res* 2011; e-pub ahead of print 16 March 2011.
5. Bhattacharya A *et al. Cancer Res* 2003; **63**: 8167–8172.
6. Benatti P *et al. Nucleic Acids Res* 2008; **36**: 1415–1428.
7. Ceribelli M *et al. Mol Cell Biol* 2008; **28**: 2047–2058.
8. Whitfield J *et al. Neuron* 2001; **29**: 629–643.
9. Hughes R *et al. Cell Death Differ* 2011; **18**: 937–947.
10. Morachis JM, Murawsky CM, Emerson BM. *Genes Dev* 2010; **24**: 135–147.
11. Imbriano C *et al. Mol Cell Biol* 2005; **25**: 3737–3751.
12. Jung MS *et al. Oncogene* 2001; **20**: 5818–5825.
13. Testoni B, Mantovani R. *Nucleic Acids Res* 2006; **34**: 928–938.
14. Tabach Y *et al. Mol Syst Biol* 2005; **1**: 0022.

15. Goodarzi H, Elemento O, Tavazoie S. *Mol Cell* 2009; **36**: 900–911.
16. Donati G, Imbriano C, Mantovani R. *Nucleic Acids Res* 2006; **34**: 3116–3127.
17. Liu Y *et al. Cell Death Differ* 2009; **16**: 847–857.
18. Oyadomari S, Mori M. *Cell Death Differ* 2004; **11**: 381–389.
19. Huang Y *et al. Cell Cycle* 2010; **9**: 328–338.
20. Ceribelli M *et al. Cell Cycle* 2006; **5**: 1102–1110.