Site-Specific Oxidative Stress Induction

Takuya Terai[1] and Tetsuo Nagano[1,*]
[1]Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-0033 Tokyo, Japan
*Correspondence: tlong@mol.f.u-tokyo.ac.jp
DOI 10.1016/j.chembiol.2007.08.002

In this issue of *Chemistry & Biology*, Kelly and colleagues describe the development of two novel ROS-generating compounds [1] that specifically localize in the nucleus or mitochondrion. Their application reveals that nuclei and mitochondria respond differently to oxidative stress, in terms of gene expression and survival pathway activation.

For all aerobic organisms including humans, molecular oxygen (O_2) is essential for survival, serving as the final electron acceptor of mitochondrial electron transport, a vital process for synthesizing ATP from ADP. Partially reduced and highly reactive metabolites of O_2 may be formed during this (and other) electron transfer reactions. These metabolites comprise superoxide anion ($O_2^{-}\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH\cdot$). These metabolites, together with other oxidizing agents such as HOCl, ozone (O_3), and singlet oxygen ($^1O_2$), are referred to as "reactive oxygen species" (ROS) [2, 3]. Since ROS could potentially damage lipids, proteins, and DNA, cells developed several defense mechanisms, which include antioxidant enzymes and targeted degradation pathways [4]. Thus, "oxidative stress" may be defined as an imbalance between ROS production and the antioxidant capacity of the cell [2]. Accumulated evidence suggests that oxidative stress is involved in a variety of human diseases, such as cancer [3], and in aging [5]. It is also well known that under severe oxidative stress, cells undergo apoptosis [4].

Location of ROS is one of the most important factors when studying oxidative stress and the cellular response to it [6]. So far, however, most studies have been conducted using oxidants that diffuse freely throughout the cell, the most well known being H_2O_2 [7]. Therefore, although it is very likely that cells rely on different mechanisms to deal with oxidative stress arising in different cellular compartments, it has not been possible to address this site-specific response.

Now, Kelly and colleagues have developed two new synthetic ROS-generating chromophore-peptide conjugates, TO-RrRK and TO-FrFK (r = *d*-arginine), that localize primarily in the nuclei and mitochondria of living cells, respectively [1]. Both of the conjugates incorporate thiazole orange (TO), a fluorescent DNA intercalator that produces $^1O_2$ upon irradiation with visible light [8]. Hence, it is the peptide moiety that plays the key role in achieving specific localization. The FrFK conjugate possesses a +3 charge and this cationic charge probably guides the molecule to mitochondria. On the other hand, according to the authors, the RrRK conjugate, which bears a +5 charge, may be too hydrophilic to access this lipophilic organelle. In any case, the specific localizations of these conjugates makes them an excellent tool for ROS researchers. As mentioned earlier, the mitochondrion is a critical subcellular component for studying ROS, since most of the physiological ROS are produced there during aerobic metabolism [2]. ROS in the nuclei are also of interest because of their potential to damage DNA [9].

After confirming that the two agents generated comparable levels of ROS in vitro, the authors proceeded to perform cell-based experiments. When cells containing TO-RrRK or TO-FrFK were exposed to light, ROS were generated in the organelle in which the conjugate was localized, and significant levels of cell death were observed (Figure 1). The results of Cy5-Annexin V/propidium iodide staining indicated that the mechanism of cell death was apoptosis, regardless of the origin of the stress. Interestingly, the compound localized to the nucleus, TO-RrRK, caused higher levels of cell death than did TO-FrFK, implying that nuclear oxidative stress is more toxic than mitochondrial oxidative stress.

Kelly and colleagues then investigated the signal transduction pathways induced by mitochondrial or nuclear oxidative stress and observed interesting differences. Experiments using various inhibitors of survival pathways that block apoptosis indicated that ERK signaling was predominant in the response to mitochondrial oxidative stress, whereas the PKC pathway was more involved in the nuclear stress response. To further elucidate the differences between the cellular response to oxidative stress originating in mitochondria and nuclei, they performed a genome-wide expression profiling experiment with a gene chip covering 47,000 genes. HeLa cells were loaded with one of the conjugates and differentially expressed genes were identified by comparing the expression levels of irradiated with nonirradiated samples. In the case of TO-RrRK, 57 transcripts were upregulated (by at least 2-fold) and 9 were downregulated upon irradiation, while in the case of TO-FrFK, 51 were upregulated and 1 was downregulated. These genes included ones known to be involved in transcriptional regulation, cell cycle regulation, apoptotic regulation, and so forth.

More importantly, the microarray experiment revealed the presence of genes that respond differently to mitochondrial and nuclear stress.

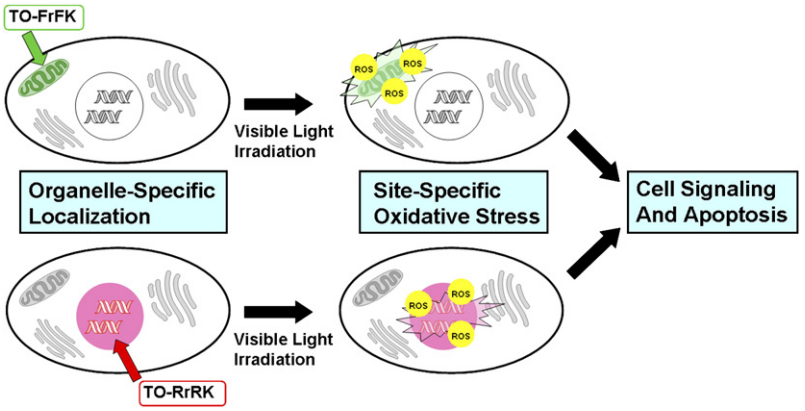

Figure 1. Organelle-Specific Induction of Oxidative Stress
The two oxidants, TO-FrFK and TO-RrRK, localize in the mitochondria and nuclei of cells, respectively. Upon visible light irradiation, these agents produce reactive oxygen species ($^1O_2$) within the cellular compartments where they are localized, inducing site-specific oxidative stress. This stress triggers gene expression, survival pathway activation, and apoptosis.

Twenty-two genes, including those of transcription factors ATF-3 and v-jun, were found to be expressed at higher levels in response to nuclear oxidative stress, whereas 5 genes, including those of the growth factors amphiregulin and epiregulin, were expressed at higher levels in response to mitochondrial stress. The results were confirmed using real-time PCR. It is noteworthy that this is the first study to link these growth factors with mitochondrial oxidative stress.

The expression of the genes that showed significant differences was also analyzed in another type of human cells, MRC-5, to determine if this pattern of response to oxidative stress was cell line-specific. Four out of eight genes tested responded in the same way in both cells, while others did not. It seems that there *are* some differences in the genomic responses of the two cell lines.

The design of ROS-generating compounds with organelle specific ocalization is not a novel concept. However, in previous studies researchers used different chromophores to achieve distinct localizations. The observed differences could not be attributed solely to the origin of ROS, which made the interpretation of the data problematic [6]. The significance of Kelly's work lies in its potential to overcome this problem. Their agents have TO as a sensitizer in common, so the intriguing results discussed above likely reflect the difference in response at the site exposed to the stress.

This study may also be instructive in the relation to photodynamic therapy (PDT). PDT is a medical treatment using light-sensitive compounds (photosensitizers) that induce cell death via ROS production [6]. The results of this study imply that therapeutic photosensitizers might be more useful if they were developed to localize specifically in the nuclei of cells.

Finally, since our own scientific interest is primarily focused on the development of fluorescence probes for biological applications, we wish to point out that, together with the site-specific ROS-generating compounds described here, molecular devices to *evaluate* the generated ROS in situ are also indispensable in order to study intracellular oxidative stress in detail. To address this question, we have very recently developed rhodamine-based ROS-reactive fluorescence probes that specifically localize in mitochondria [10], in contrast to previous fluorescein-based ones that spread throughout the cell [11]. The combined use of Kelly's photosensitizers and our probes may open up a new dimension in studies on oxidative stress.

REFERENCES

1. Mahon, K.P., Potocky, T.B., Blair, D., Roy, M.D., Stewart, K.M., Chiles, T.C., and Kelley, S.O. (2007). Chem. Biol. *14*, this issue, 923–930.
2. Thannickal, V.J., and Fanburg, B.L. (2000). Am. J. Physiol. Lung Cell. Mol. Physiol. *279*, L1005–L1028.
3. Wiseman, H., and Halliwell, B. (1996). Biochem. J. *313*, 17–29.
4. Martindale, J.L., and Holbrook, N.J. (2002). J. Cell. Physiol. *192*, 1–15.
5. Finkel, T., and Holbrook, N.J. (2000). Nature *408*, 239–247.
6. Moor, A.C.E. (2000). J. Photochem. Photobiol. B *57*, 1–13.
7. Aikawa, R., Komuro, I., Yamazaki, T., Zou, Y.Z., Kudoh, S., Tanaka, M., Shiojima, I., Hiroi, Y., and Yazaki, Y. (1997). J. Clin. Invest. *100*, 1813–1821.
8. Wittenhagen, L.M., Carreon, J.R., Prestwich, E.G., and Kelly, S.O. (2005). Angew. Chem. Int. Ed. *44*, 2542–2546.
9. Croteau, D.L., and Bohr, V.A. (1997). J. Biol. Chem. *272*, 25409–25412.
10. Koide, Y., Urano, Y., Kenmoku, S., Kojima, H., and Nagano, T. (2007). J. Am. Chem. Soc., in press.
11. Setsukinai, K., Urano, Y., Kakinuma, K., Majima, H.J., and Nagano, T. (2003). J. Biol. Chem. *278*, 3170–3175.

