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Posters

Non-mammalian NO

P010. The nitric oxide response of *Escherichia coli*

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In enteric bacteria, such as *Escherichia coli* and *Salmonella typhimurium*, nitric oxide (NO) is made endogenously at low concentrations, as a by-product of anaerobic nitrate and nitrite respiration. These bacteria have evolved specific defense mechanisms to counteract NO, and regulatory proteins that sense NO provide the primary response by controlling the expression of genes encoding various enzymes that reduce or oxidize NO to less toxic species. Thus, NO is analogous to superoxide (as a toxic by-product of a normal metabolic process), and I speculate that the enzymes that act on NO have an analogous physiological role to that of superoxide dismutase. Pathogenic strains also encounter much higher concentrations of NO in host phagocytic cells, where NO is made from arginine by the inducible NO synthase. There is increasing evidence that enzymes that reduce or oxidize NO, and their regulators, have been exploited by pathogens as a means of resisting phagocyte-derived NO. I will review the various mechanisms by which *E. coli* senses and responds to NO, and will describe the mechanism of the best characterized NO sensor, NorR. I will go on to describe our recent discovery of a novel *E. coli* regulatory protein that mediates a response to NO.

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P011. Plant gametophyte models for understanding the connection between calcium and nitric oxide signaling in plants

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Nitric oxide (NO), a ubiquitous signaling molecule in animals, has been implicated in numerous plant signaling systems. Our work has focused on understanding the role of NO and cGMP in two model plant gametophyte systems, *Lilium longiflorum* pollen tubes, and *Ceratopteris* fern spores. Both of these haploid, single cell systems are known to be developmentally dependent on localized calcium gradients, which actively drive cellular polarity. Lily pollen tubes were grown in vitro and showed a negative tropism when challenged with an artificially generated NO point source (1–3 μm). Similarly, we found that if *Ceratopteris* fern spores are exposed to NO generating compounds, then gravity dependent cellular development and polarity were disrupted in a dose dependent manner. Both of these effects were inhibited by the NO scavenger PTIO. In pollen tubes we imaged NO localization, using the fluorophore DAF-2 and confocal microscopy, and were able to determine that the distribution pattern was consistent with peroxisomes as the NO source. We also found that cGMP is the putative downstream messenger of NO in both of these systems. In Lily pollen tubes, we were able to show that the application of the phosphodiesterase inhibitor sildenfil citrate (Viagra) to pollen tubes could induce growth responses in the presence of a weak suboptimal NO source that previously failed to induce reorientation without the drug. In *Ceratopteris* Viagra and guanylate cyclase inhibitors also disrupted cellular polarity dynamics in dose response experiments. These results demonstrate NO/cGMP signaling cascades are active components in calcium dependent signaling and cellular polarity in plant systems.

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P012. Conventional screening method of short-term in vitro assay for nitric oxide scavenger reagents

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The important physiological roles of nitric oxide(NO) suggest that donation of NO may be useful for the treatment of several states, and bioactivity of NO was indistinguishable from that of endothelium derived relaxing factor. Some pharmacological NO donors, that they may serve as natural strange and transport forms for bioregulatory NO and currently in use for the biological generation of NO. Other important feature of NO is a mutagenic compounds that can cause mutations in bacteria as well as chromosomal aberrations in rat primary lung cells and it is best known as a toxic reactive free radical. NO is emerging as an important mediator of cytotoxicity and/or mutagenicity.

In order to develop a possible in vitro screening model of scavenger against NO, we begin to explore the potential role of treatment scavenging of malignant NO production. Chang liver cells (human derived) from in DMEM were cultured for 3 days before treatment. NOR1(NO donor) was added into culture dish and incubated for 1 h under CO_2 incubator as control.

For screening assay, test samples to culture dish were added before 1 min of NOR1 treatment. Transform cells were observed under light-microscopy (×100) without stained.

All observed cells count for more than 250. The inhibitory ratio was then calculated for arrangement of data. Antioxidative reagents tested showed moderate inhibitory effects on NOR1 activation. In present paper, we designed a short-term in vitro assay for detecting NO scavengers, and test is simple to perform, reproducible and should be applicable for mass-screening of useful substances in our environment.

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