phosphoprotein, and the phosphorylation of the carboxy terminus by protein kinases, such as protein kinase C (PKC), Src, and MAPKs, in the regulation of GJIC has been well documented (Solan and Lampe 2005). Although phosphorylation of gap junctions is known to regulate the function, assembly, internalization, and degradation of this protein complex, the alteration of connexin phosphorylation by protein kinases, such as MAPKs, does not necessarily dysregulate gap junction function (Hossain et al. 1999), nor does the activation of protein kinases (i.e., MAPK) alter the phosphorylation status of connexins (Upham et al. 2008).

This was also true for PFOA, which clearly activated ERK-MAPK (Figure 3) but did not induce a change in the phosphorylation pattern of Cx43 as previously determined by Western blot analysis (Upham et al. 1998). Whether or not gap junctions are phosphorylated, several compounds (i.e., growth factors, lindane, lysophosphatidic acid, 12-*O*-tetradecanoylphorbol-13-acetate, and cannabinoids) are known to inhibit GJIC through a MEK-dependent pathway (Komatsu et al. 2006; Mograbi et al. 2003; Rivedal and Opsahl 2001; Upham et al. 2003). Although many compounds activate MAPKs, such as p38 and ERK, the mechanism of inhibiting GJIC by many of these compounds is independent from these MAPKs (Machala et al. 2003; Upham et al. 2008).

Our results indicated that PFOA activated ERK in F344 WB rat liver epithelial cells within 5 min, and this time period is within the interval required for the inhibition of GJIC by PFOA in this cell line. PFPeA, which does not inhibit GJIC in this cell line (Upham et al. 1998), also did not activate ERK. Preincubation of these cells with an MEK inhibitor, U0126, partially prevented PFOA from inhibiting GJIC, indicating that PFOA-induced modulation of GJIC was not solely dependent on the ERK pathway.

Recently, PC-PLC has been implicated in the dysregulation of GJIC in response to toxicants that regulate GJIC through an MEK-independent mechanism (Machala et al. 2003; Upham et al. 2008). Preincubation of F344 WB cells with the PC-PLC inhibitor D609 also partially prevented PFOA from inhibiting GJIC. These results suggest that PFOA is regulating GJIC through multiple cellular mechanisms. This becomes more apparent as the dose of PFOA is increased resulting in the inhibition of GJIC at a high dose of 120 μM that depended on neither PC-PLC nor MEK. However, maximum inhibition of GJIC by PFOA, which was around 80 μM, was very dependent on the activity of both MEK and PC-PLC. This was further apparent from the experiment where cells were pretreated with a combination of both D609 and U0126, resulting in almost complete recovery of GJIC. The activation of ERK and PC-PLC will not only control gap junction function but is known to alter gene expression, leading to various pathologies, including cancer. The function of PC-PLC in tumorigenesis has not been extensively studied, yet there are significant reports indicating that PC-PLC does play a very significant role in cancer (Cheng et al. 1997). The ERK pathway has been extensively characterized and is the most understood of the MAPK pathways (Denhardt 1996) and is a key pathway of carcinogenesis (Roberts and Der 2007).

PFOA, but not perfluorobutyrate, is known to induce oxidative stress in the livers of rats, as indicated by 8-hydroxydeoxyguanosine formation (Takagi et al. 1991), and redox mechanisms are known to commonly play a role in gap junction function (Upham and Trosko 2009). These oxidative signaling effects could be site-directed redox regulations of specific regulatory proteins or from general oxidative effects (Upham and Trosko 2008). Recently, we reported that the antioxidant resveratrol prevented inhibition of GJIC by dicumylperoxide but not by benzoylperoxide (Upham et al. 2007). Dicumylperoxide, but not benzoylperoxide, inhibits GJIC through a PC-PLC–dependent mechanism (Upham et al. 2007). Similar to dicumylperoxide, we showed that resveratrol prevented inhibition of GJIC by PFOA to a greater level than either D609 or U0126 alone, but similar to the level of GJIC recovery seen when cells were pretreated with both D609 and U0126. These results indicate the possibility that PFOA dysregulates GJIC through both MEK and PC-PLC and that protection of GJIC by resveratrol is potentially through oxidative signaling events controlling both MEK and PC-PLC. Beyond the implication of redox mechanisms of the resveratrol experiment, this antioxidant is regularly consumed by humans and is found in high concentrations in red wine and peanut products (Sobolev and Cole 1999; Wang et al. 2002), and thus may have some relevance to the health of humans that may be exposed to environmental toxicants, such as PFOA. Chemopreventive effects of resveratrol are known to inhibit initiation, promotion, and progression of tumors (Signorelli and Ghidoni 2005). Thus, resveratrol could potentially contribute to a protective effect in humans exposed to PFOA by significantly blocking PFOA from inhibiting GJIC.

The addition of Asc-2-P or Nac partially reversed the inhibitory effects of PFOA on GJIC, similar to that of resveratrol. In contrast, DTT did not prevent PFOA from inhibiting GJIC, indicating that the oxidative events controlling PC-PLC and Mek are not thiol based. The exposure of F344 WB cells to PFOA for 2 days showed no adverse effects on cell morphology, and they communicated normally after PFOA was removed from the medium (Figure 6), which implicates that the PFOA-induced oxidative events are not killing the cells. These results suggest that general oxidative processes are involved in PFOA-induced inhibition of GJIC and that health benefits could potentially be attained by the consumption of many antioxidant rich foods, particularly in individuals deficient in antioxidants. Moreover, the reversible properties of PFOA-induced inhibition of GJIC are consistent with the known reversible nature of tumor promoters in two-stage carcinogenesis model systems (Trosko and Upham 2005). These results also indicate that reversing the effect of PFOA on GJIC after a simple washing of the treated cells with PBS demonstrates that PFOA is not covalently or tightly bound to the cell. The effect of PFOA on GJIC was probably not a consequence of directly interacting with the gap junction proteins because the inhibition of MAPK and PC-PLC both prevented the GJIC effect. Possibly PFOA interacted with these two proteins or interacted with a signaling protein or receptor even further upstream.

In conclusion, the *in vitro* assay system used to assess the effects of PFOA and PFPeA on GJIC predicted the *in vivo* results of GJIC from rats treated with these compounds. GJIC plays a vital role in maintaining tissue homeostasis, and disruption of gap junction function can lead to diseased states such as tumorigenesis. These results are similar to other tumor-promoting compounds tested in both an *in vitro* and *in vivo* assay system. Although there are several mechanisms by which environmental compounds might promote an initiated cell, such as through peroxisome proliferator activated receptors or protein kinase C, the disruption of normal intercellular communication is an essential event of multiple tumorigenic mechanisms (Trosko and Upham 2005) and serves as a central biomarker to assess the epigenetic toxicity of contaminants (Rosenkranz et al. 1997; Trosko and Upham 2005), as well as to assess the potential antitumorigenic health benefits of nutrition based food products (Trosko and Upham 2005).

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