Published in final edited form as:

Mol Cell Endocrinol. 2012 May 6; 354(0): 60–62. doi:10.1016/j.mce.2011.11.028.

Environmental signaling and reproduction: A comparative biological and chemical perspective

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Abstract

Reproduction is a critical element of life. Self-propagation in all living organisms ranging from bacteria to humans involves numerous common strategies. Underlying all reproductive strategies is the essential need for signaling molecules to initiate and maintain the process. In this paper we use comparative biological and chemical approaches to explore the origins and distribution of estrogen signaling as a pathway common to many life forms. In the process we illuminate the mechanisms whereby environmental agents alter reproduction and development. These mechanisms involve altered signaling pathways within cells and shifts in the targets of the signaling pathways to include regulators of gene transcription normally associated with other pathways. We also stress the role of signal cross talk in mediating hormone action.

Keywords

Environmental signaling; Estrogen receptor; Estrogenic; Reproduction; Endocrine disruption; DDT; BPA; Soy isoflavone; Phytoalexin

Reproducing oneself is one of the most critical events in the life process of all species. In vertebrates, there are numerous signaling molecules that initiate and maintain reproduction. From the standpoint of comparative endocrinology it has been surprising to learn that the signaling molecules and, in many cases, the strategies are more similar than different. Signal transducing factors, or receptors (Rcs), are similar, but distinguishable across species while the signaling molecules are identical. The offshoot is that signals have evolved at different levels of complexity, but a similar base plan (cybernetic feed back) is seen at almost all levels; this may explain why various natural molecules, derived from most parts of the

phylogenetic system can activate signaling systems; synthetic chemicals can participate in the signal activation system even though the structures may differ widely.

Control of critical life functions such as reproduction, homeostasis, metabolism and organismal integration and regulation is accomplished by integrated networks of signaling systems that include effector signals and their cognate transducers (Crews and McLachlan, 2006; McLachlan, 2001; Soto et al., 2009). In the endocrine system these signals and sensors are called hormones and receptors. Other systems include immune, nervous, cardiovascular and digestive. While little is known about the interaction of external environmental factors with the latter four systems, a great deal of information has accumulated regarding the environment and the endocrine system. In fact, one might consider that environmental signaling, that is the explicit interaction of environmental factors with the endocrine system, has been discovered to be a major way that the external environment interacts with the internal one. Chemicals derived from natural products in plants, invertebrates and industrial sources can mimic the action of endogenous hormones. These include, apparently, sex steroids, thyroid hormone and others. Of these, the ovarian steroid hormone, estrogen, seems to be the most often mimicked or blocked by environmental factors (Tilghman et al., 2010).

Results of inadvertent experiments observed in laboratories provide strong examples of the impact environmental compounds may have on the reproductive endocrinology of mammals. One of the earliest reports is that of uterine stimulation in mice exposed to a pesticide as a result of disinfection of the space in which they were housed; the active uterotrophic agent was found to be the DDT derivative, methoxychlor (Tullner, 1961). Since this early report, industrial compounds with diverse chemical structures have been found to exert weak estrogenic activities. Pollutants such as DDT, PCB and kepones are in this class. These compounds are chlorinated, and the location of chlorine atoms seems to be a determining factor in the hormonal activity of each. For example, o,p-DDT is more active as an estrogen than is p,p-DDT (Klotz et al., 1996). The position of chlorine atoms and hydroxyl groups in a series of hydroxylated PCBs determines the estrogen receptor alpha binding and estrogenic activities that range from biologically significant to undetectable (Korach et al., 1988). More recently, Hunt et al. (2003) reported estrogen-like effects on the ova of mice housed in damaged plastic cages. Through a series of careful experiments she demonstrated that the plastic component, bis-phenol A (BPA) that had been released from the damaged cages was mimicking the action of estrogen treatment on the ovulating mice, resulting in meiotic aneuploidy. Numerous other examples of the estrogenic activities of environmental agents can be found (McLachlan, 2001).

Estrogen-like compounds also occur naturally in legumes such as soy (Burow et al., 2001; Oberdorster et al., 2001) and in some fungi such as *Fusarium solani* (Gilbert, 1989). In the first case, the estrogenic compound is an isoflavone structure while the second is a resorcylic lactone – neither one a steroid. Taken together, compounds with hormonal activity are called endocrine disrupting chemicals (EDCs). While some toxicological effects are known (feminization of fish and subsequent reproductive dysfunction) (Tilghman et al., 2010) others are potential (early puberty in girls or decreased reproductive function in men) (Li et al., 2010). EDCs also provide unique windows to the origins and environmental integration of signals and the signaling systems of living organisms.

Plants are a rich source of chemicals that elicit hormone like responses in the vertebrates that ingest them. Among plants, naturally occurring isoflavones (genestein) (Boue et al., 2003), coumarins (coumestrol) (Nelson et al., 1984) or stilbenes (resveratrol) (Boue et al., 2009) elicit estrogenic responses usually associated with hydroxy-steroids. In fact, some chemical signals from plants result in reciprocal responses from other organisms – symbiotic signals.

For example, Sharon Long's lab has demonstrated that leguminous chemicals secreted from the root tip of the plant interact with specific transcription factors on bacterial genes resulting in the production of unique bacterial/plant structures (Peters et al., 1986). Nodulation thus takes place within the root of the plant and is the primary structure for nitrogen fixation in leguminous plants. Our lab established a system to probe the interaction between the chemical environment and symbiotic signaling (Fox et al., 2001a). Our system utilized the symbiotic signaling between the alfalfa plant, *Medicago sativa*, and the soil bacterium, *Sinorhizobium melliloti*. The activating signal from alfalfa, luteolin (a compound that exhibits weak estrogenic activity) (Collins-Burow et al., 2000) interacts with a transcription factor in *S. melliloti* resulting in the production of key gene products involved in the nodulation response. We wondered if the symbiotic signaling system between plant and bacteria would be susceptible to disruption by synthetic environmental chemicals in a manner similar to endocrine disruption. We found that at environmentally relevant concentrations, EDCs predictably altered the Nod gene response of the bacteria (Fox et al., 2001a,b). Just as with endocrine disruption, symbiotic signaling disruption has functional consequences. Blocked signals result in poor nodulation, lack of nitrogen fixation and diminished plant growth (Fox et al., 2007). The consequences for global crop yield could be catastrophic if unchecked.

That legumes produce weak acting estrogenic compounds has been known for a long time (Burow et al., 2001; Collins-Burow et al., 2000; Nakari, 2005; Tonetti et al., 2007). In fact, soy based estrogenic isoflavones have been studied in post menopausal women (Baird et al., 1995) and have been considered important to the beneficial health effects of soy based diets in women. Those benefits include reduction in breast cancer risk (Nagata, 2010; Velentzis et al., 2008) and improved cardiovascular health (Mulvihill and Huff, 2010; Siow and Mann, 2010). In the symbiotic signaling system of legumes and rhizobial bacteria, we found that the positive Nod gene-activating signal, luteolin, could be offset by a negative suppressing signal, chrysin (Oberdorster et al., 2001; Collins et al., 1997). This observation encouraged us to look for signaling molecules that might antagonize the estrogenic signals already studied. Our laboratory reported that soybeans grown under stress also produce a chemical that functions as an antiestrogen in mammalian systems (Burow et al., 2001). Rather than a phytoestrogen, the chemical, glyceollin, is a phytoalexin, a compound that acts as a fungicide. Glyceollin antagonizes estrogen action at the receptor level (Burow et al., 2001), impedes growth of human breast cancer xenographs in mice (Salvo et al., 2006) and reduces the response of the rhesus monkey breast to estrogen (Wood et al., 2006).

Over the last two decades mechanisms for estrogen action have been broadened to include cellular signaling pathways in addition to the classical nuclear estrogen receptor (ER). Our laboratory showed some time ago that both estrogen and membrane associated growth factors such as EGF and TGF α could elicit similar effects in the uterus of intact mice (Bossert et al., 1990; Nelson et al., 1992) or explants of mouse uterus carried in the kidney capsule of mice (Ignar-Trowbridge et al., 1992). Subsequently, we showed that estrogen and EGF demonstrated cross talk in their signaling in cell culture of uterine epithelial cells (Ignar-Trowbridge et al., 1995). We showed that in uterine cells the estrogen like action of EGF required a functioning ER α (Ignar-Trowbridge et al., 1995). It was shown in mice lacking the ER α gene that EGF effects were abolished (Curtis et al., 1996). At the same time, cross talk between peptide hormone receptors and the steroid hormone receptors was demonstrated for dopamine and progesterone receptor (PR) (Power et al., 1991) and IGF-1 and ER α (Aronica and Katzenellenbogen, 1993). We added evidence for the importance of signal cross talk and receptor convergence by showing that the cell survival pathway associated with PI-3 kinase and AKT requires a functioning ER α for action (Bratton et al., 2010; Burow et al., 2000). The nuclear complex associated with ER α may be an important site of convergence for pathways involved in proliferation, survival and differentiation. For

a short review of rapid estrogen signaling and multiple pathways for multiple disease endpoints, see (Tilghman et al., 2010). As the study of estrogen signaling has matured, the “classic” or nuclear receptor action of estrogen has been called “genomic” while the “alternative” or membrane associated action has been referred to as “non-genomic”. Since, as stated above, various signaling pathways converge at the receptor or receptor associated complexes, we think that “non-genomic” is inaccurate, and we prefer the terms Nuclear Initiated Steroid Signaling or NISS and Membrane Initiated Steroid Signaling or MISS (Nemere et al., 2003). We are aware, however, that many of the environmental agents capable of inducing estrogenic actions by either or both of these pathways are not steroids.

The various mechanisms employed by EDCs raise very interesting possibilities for environmental endocrine science. By offering multiple signaling pathways, diverse chemicals may use all or any combination of the pathways within cells. Thus, DDT, DES, BPA or estradiol may utilize different combinations while retaining an essential aspect of estrogenicity. To explore this hypothesis, we used the estrogenic and non-estrogenic forms of DDT to show that DDT can elicit changes in estrogen target cells through ER independent mechanisms. Our results showed that DDT and its congeners can induce AP-1 mediated transcription in cells with or without ER (Frigo et al., 2002). DDT will also activate the p38 α and ERK1/2 signaling pathways in the absence of either estradiol or ER or both (Frigo et al., 2004). DDT apparently activates transcription at multiple DNA response elements through p38 α associated phosphorylation and recruitment of the ER co-activators GRIP1 (Frigo et al., 2006) and p300 (Bratton et al., 2009). Co-regulators are critical components of the estrogen receptor transcriptional complex (O’Malley and Kumar, 2009). Thus, in a search for the likely targets for estrogenic chemical signal convergence we should consider the recruitment of co-regulators to the ER complex by multiple estrogenic substances.

As an approach to understanding the estrogenic action of chlorinated hydrocarbons like DDT, we again utilized comparative biology and chemistry. The naturally chlorinated hydrocarbon, DIF-1, a biphenolic compound that is produced by the slime mold *Dictostelium discoideum*, is an endogenous reproductive signal (Early, 1999). In the mold, DIF-1 functions as a pro-differentiation signal. In mammalian cells, we found that DIF-1 is an anti-estrogenic and anti-proliferative compound through its unique ER gene regulation ability (Bratton et al., manuscript in preparation).

In the emerging field of environmental endocrine science, the use of comparative approaches is leading to new insights into the potential mechanisms of action of environmental agents as well as shedding new light on endogenous sex steroids.

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