Sex as a response to oxidative stress: the effect of antioxidants on sexual induction in a facultatively sexual lineage

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1. INTRODUCTION

Despite over 30 years of research, the origins and evolutionary roles of sex remain a fundamental problem in biology. Although in many lineages sex is an obligatory part of the life cycle, in prokaryotes and many lower eukaryotes, sex is facultative, occurs in response to stress and often involves the formation of a stress-resistant dormant form. The proximate and ultimate causes of the connection between stress and sex in facultatively sexual lineages are unclear. Because most forms of stress result in the overproduction of cellular reactive oxygen species (ROS), we address the hypothesis that this connection involves ROS and possibly reflects the ancestral role of sex as an adaptive response to the damaging effects of stress-induced ROS (i.e. oxidative stress).

Here, we report that two antioxidants inhibit sexual induction in a facultatively sexual species—the multicellular green alga, Volvox carteri. Furthermore, the nature of the sex response and the effect of an iron chelator on sexual induction are consistent with sex being a response to the DNA-damaging effects of ROS. In addition, we present preliminary data to suggest that sex, cell-cycle arrest and apoptosis are alternative responses to increased levels of oxidative stress.

2. MATERIAL AND METHODS

Volvox carteri, female strain, was kindly provided by Dr David L. Kirk (University of New Brunswick). Synchronous cultures of asexual
females were grown in standard Volvox medium, on a 16 L : 8 D cycle (Kirk & Kirk 1986). Cultures were subjected to a 42.5 °C-heat stress for 2 hours (Kirk & Kirk 1986). The medium in which somatic cells released the sexual inducer (i.e. the conditioned medium) was collected 18–20 hours after the heat-shock and used to induce sex in unstressed females at a density of one female per 2 ml (Kirk & Kirk 1986). The two antioxidants, catalase (Sigma; 12 800 U mg⁻¹) and CuDIPSH (Aldrich; 10 mM stock in acetone), and the Fe chelator, 2,2′-dipyridyl (Sigma; 20 mM stock in water) were added to cultures at final concentrations of 5 µg ml⁻¹, 2 µM and 20 µM, respectively.

3. RESULTS AND DISCUSSION

We heat-shocked V. carteri cultures of five females per ml, in the absence or presence of either catalase or CuDIPSH. Three days later, the percentage of sexual progeny relative to total progeny was calculated. If sexual induction in V. carteri is mediated by ROS, agents that remove ROS should diminish the sexual response. Consistent with this prediction, both antioxidants reduced (by up to 100%) the percentage of sexual progeny (figure 1a). As sexual development is, ultimately, triggered by the sexual inducer, we tested the effect of the antioxidants on the inducer’s mode of action alone. We used conditioned media from heat-shocked cultures of five females per ml to induce sex in unstressed cultures, in the absence and presence of the antioxidants. As expected if ROS were involved in this process, the two antioxidants diminished the effectiveness of the inducer in triggering sexual development (figure 1b).

Interestingly, the efficiency of the antioxidants in reducing the sex response during the heat-shock experiments is higher than that observed when sexual development was induced via conditioned media (figure 1). The difference suggests that in the former case, in addition to inhibiting the inducer’s mode of action (i.e. the 50% inhibition observed when sex was induced using conditioned media), the antioxidants have also diminished the production of the sexual inducer. This argues for the participation of ROS in both the production (by the somatic cells) and the activity of the inducer in triggering sexual development in gonidia.

As the effect of H₂O₂ is dependent on its dose, we have investigated the sex response as a function of algal density, inducer dose and stress intensity. We have heat-stressed cultures of increasing density and noted that the sex response is strongly affected by the density of the culture, in a nonlinear fashion: the sex response is high at low algal densities, then drops as much as 60–70%, after which the response increases again with increasing density (figure 3a). A nonlinear relationship was also observed when

(i) conditioned media collected from heat-shocked cultures of increasing algal density and
(ii) 1 : 1 serial dilutions of conditioned media collected from a culture of 20 females per ml were used to induce sex in unstressed cultures (figure 3b, c).

Interestingly, the relationship between inducer dose and sex response (figure 3c) is very similar to the pattern
Figure 3. Sex response as a function of algal density, inducer dose and level of stress. The error bars represent sample standard deviations using the nonbiased or ‘n – 1’ method; each point represents the average of three independent experiments. When only one experiment is shown, it indicates the trend observed in more than three independent experiments. (a) Sex response as a function of algal density; the percentage of sexual progeny was calculated relative to total progeny (ca. 500 individuals). (b) Sex response to conditioned media collected from heat-shocked cultures of increasing algal density; conditioned media were used to induce sex in unstressed females at a density of one female per 2 ml; the percentage of sexual progeny was calculated relative to the total progeny (ca. 200 individuals). (c) Sex response as a function of inducer dose; conditioned medium from a heat-shocked culture of 20 females per ml was 1:1 serially diluted and used to induce asexual females at a density of one female per 2 ml. (d) The effect of the Fe^{2+} chelator, 2,2’-dipyridyl, on the sex response to conditioned media from heat-shocked cultures of increasing algal density; the circles and squares denote the sex response in untreated and treated media, respectively. (e) Heat-induced sex response (squares) and cell-cycle arrest of gonidia (circles) as a function of algal density; the percentage of arrested gonidia was calculated as the percentage of females in which more than half of the gonidia have not developed into embryos by the second day after exposure to heat-shock. (f) Heat-induced (i.e. 43.5 °C for 120 min) apoptosis in gonidia; (i) complete dissolution of the gonidia; (ii) surviving gonidia develop into sexual embryos.
observed for cellular responses to increasing doses of DNA-damaging H$_2$O$_2$: the responses are high at low H$_2$O$_2$ doses, then drop off, to increase again and become roughly independent of concentration at higher H$_2$O$_2$ doses (Imlay et al. 1988). The damaging effect of H$_2$O$_2$ is attributed to hydroxyl radicals (and other unknown antioxidants) formed during the Fenton reaction (Fe$^{2+}$ + H$_2$O$_2$ + H$^+$ → Fe$^{3+}$ + H$_2$O + HO$^*$) and to the fact that at distinct H$_2$O$_2$ doses, Fe$^{2+}$ produces oxidants with different affinities for DNA and different DNA-damaging effects (Imlay et al. 1988; Luo et al. 1994). As Fe$^{2+}$ chelators can only remove Fe$^{2+}$ ions that are loosely associated with DNA (and which are responsible for the DNA-damaging effect of low H$_2$O$_2$ doses), we tested the effect of the Fe$^{2+}$ chelator, 2,2’-dipyridyl, on the sex response to conditioned media collected from heat-stressed cultures of increasing algal densities. The chelator was effective in inhibiting sexual induction, but its effectiveness was dependent on the density of the culture from which the media was collected (figure 3d), in a fashion that opens up the possibility of an Fe$^{2+}$-mediated H$_2$O$_2$–DNA interaction in the inducer’s mode of action. A direct correlation between the sexual inducer and DNA damage in V. carteri would not be without precedent: at high concentrations, the yeast sex inducer increases the production of intra-cellular ROS in its target cells, inducing DNA damage and apoptosis (Severin & Hyman 2002).

It is noteworthy that the unusual H$_2$O$_2$ dose-dependent response discussed here has been shown to be associated with preferential DNA nicking; at low doses of H$_2$O$_2$, purine–thymine–guanine–purine (RTGR) sites are preferentially nicked, whereas at higher concentrations, purine–guanine–guanine–guanine (RGGG) sites are preferred (Rai et al. 2001). Furthermore, RTGR sequences are known to be parts of required motifs in regulatory elements of genes involved in responses to oxidative stress. Thus, it is tempting to speculate that in V. carteri, sex might be induced by DNA nicking of sequences present in regulatory regions of genes associated with sexual induction.

Various cellular processes are known to be responses to increasing levels of ROS (and often DNA damage), including cell-cycle arrest and apoptosis. Our observation that, in V. carteri, sex is part of a series of cellular responses to increasing levels of stress and ROS argues further for the involvement of ROS in sexual induction. At densities of higher than five females per ml, the usual sex-inducing heat-shock triggers a transitory cell-cycle arrest in most or all gonidia; this is followed by a drastic decrease in the percentage of sexual progeny (figure 3e) as these arrested gonidia develop into asexual rather than sexual embryos. Furthermore, a slight increase in the heat-stress duration or intensity (e.g. 42.5 °C for 3 hours, or 2 hours at 43.5 °C) results in many or all gonidia undergoing apoptosis (as evidenced by the complete dissolution of gonidia within several hours (figure 3f) and by the DNA laddering effect indicative of apoptosis (A. M. Nedelcu and R. E. Michod, unpublished data)), while the surviving gonidia develop into sexual embryos (figure 3f). These results suggest that sex, cell-cycle arrest and apoptosis are alternative responses to increased levels of stress.

The evolution of sex remains one of the great, unsolved problems in biology. If the participation of ROS in sex proves to extend to other types of stress and other lineages (A. M. Nedelcu and R. E. Michod, work in progress), these findings will impact greatly on our understanding of the ecological and adaptive significance of sex.

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