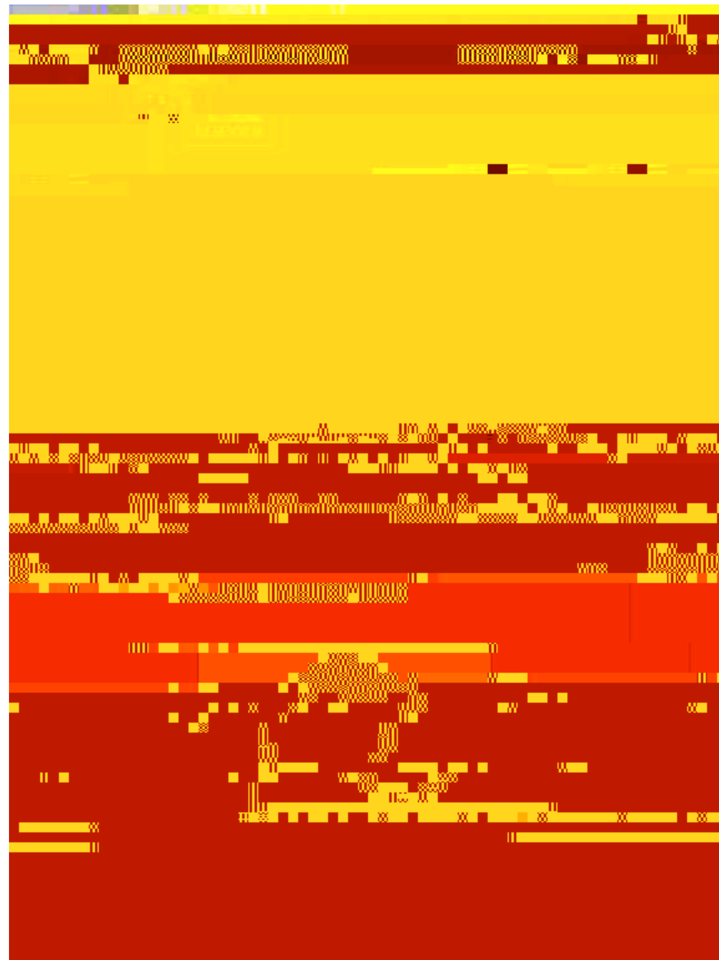
Provided for non-commercial research and educational use only.
Not for reproduction or distribution or commercial use.



This article was originally published in a journal published by Elsevier, and the attached copy is provided by Elsevier for the author's benefit and for the benefit of the author's institution, for non-commercial research and educational use including without limitation use in instruction at your institution, sending it to specific colleagues that you know, and providing a copy to your institution's administrator.

All other uses, reproduction and distribution, including without limitation commercial reprints, selling or licensing copies or access, or posting on open internet sites, your personal or institution's website or repository, are prohibited. For exceptions, permission may be sought for such use through Elsevier's permissions site at:

http://www.elsevier.com/locate/permissionusematerial

Available online at www.sciencedirect.com
ScienceDirect

Reproductive T

Author's personal copy

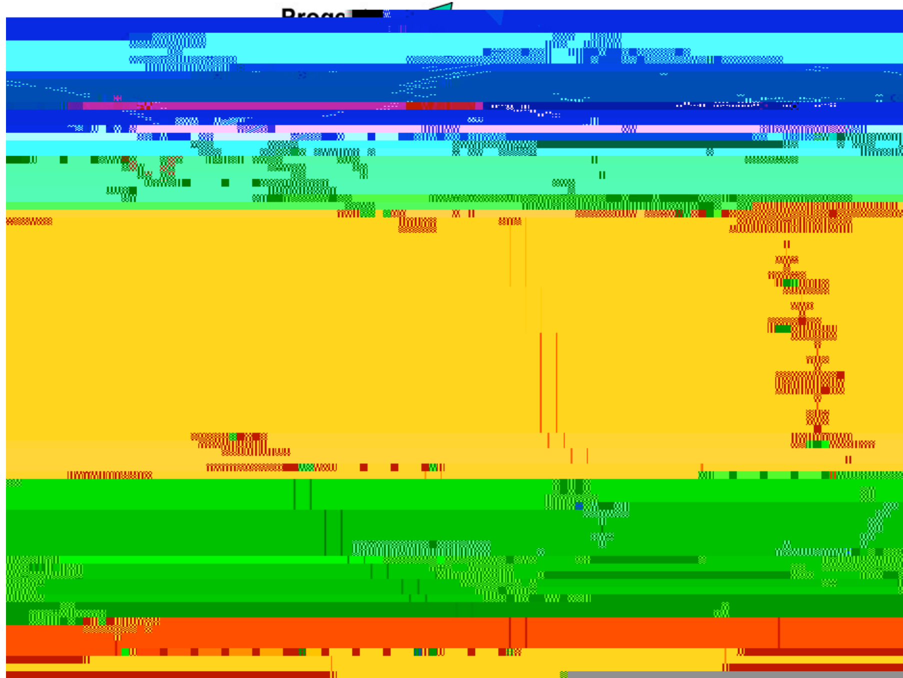


Fig. 1. Brief model of progesterone-stimulated meiotic maturation. Arrows denote activ

Author's personal copy

2. M

2.1. O c e a

Ovariectomies were performed on anaesthetized adult *Xe ae* (Xenopus Express) through a small ventral incision. Oocytes were enzymatically defolliculated by gentle rocking at room temperature (24 C) for approximately 1 h in collagenase (2 mg/ml), dispase (1.2 mg/ml), 1× modified Barth's saline (MBS) (88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.41 mM $CaCl_2 \cdot H_2O$, 0.82 mM $MgSO_4 \cdot 7H_2O$, 2.4 mM $NaHCO_3$, 10 mM HEPES, pH 7.4). After rinsing extensively in 1× MBS, stage VI oocytes (the largest) were manually selected and allowed to equilibrate overnight in 1× MBS, pH 6.8 at 17 C.

2.2. O c e c ba c ec a d c d ec

Oocytes were incubated at room temperature in 1× MBS, pH 6.8 supplemented as indicated with 10 M progesterone (dissolved in ethanol) (Sigma, St. Louis, MO);

Author's personal copy

reagents according to the manufacturer's instructions (Amersham Biosciences Corp.), and autoradiography. Five oocytes from each treatment were homogenized and typically 0.5–1

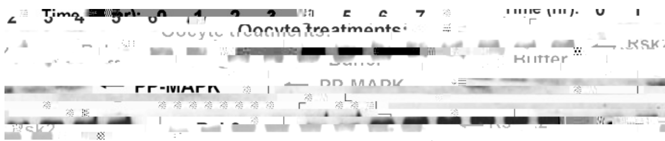
Author's personal copy

Under these late conditions, Mos protein was expressed and P-Cdc2 levels were visibly reduced. These data suggest that 2,4-D does not require PKA or factors upstream of PKA to block maturation but targets a factor or factors(s) downstream of PKA that is required during at least the first 4–5 h of the signaling pathway (however, see below).

3.2. 2,4-D e e , e e e, f M , a e a , a a e e

It is well established that both progesterone and PKI-induced maturation are dependent on translation [14,16,30]. Mos expression itself is regulated by both translational control and protein stabilization [36]. Preliminary experiments indicated that the level of Mos protein present in mature oocytes was unaffected by prolonged exposure to 2,4-D (data not shown). However, 2,4-D did block Mos expression during the first 4 h of progesterone exposure (data not shown). These data suggested that 2,4-D prevented *de* synthesis of Mos rather than promote protein degradation. Progesterone stimulates cytoplasmic polyadenylation and translation of Mos mRNA. To test whether 2,4-D inhibits this mechanism, oocytes were microinjected with radiolabelled transcripts of Mos 3 untranslated region (UTR), which contains the polyadenylation signals, and exposed to progesterone and 2,4-D. Total RNA was subsequently isolated from oocytes, fractionated by denaturing gel electrophoresis and visualized by autoradiography. Poly(Ao9e

Author's personal copy



Author personal copy

Fig. 6. Reduction of H1 kinase activity in MII oocytes exposed to 2,4-D. (a) Oocytes, all from a single female, were treated as indicated on the left and withdrawn hourly, as marked above each panel. Homogenates were prepared and assayed for H1 kinase activity. Proteins were fractionated by electrophoresis and visualized by autoradiography. Arrows indicate when 2,4-D was added to progesterone-treated samples: = 0 h, concomitant to progesterone, and at = 5 h, after oocytes had undergone GVBD (data not shown) and were

Author's

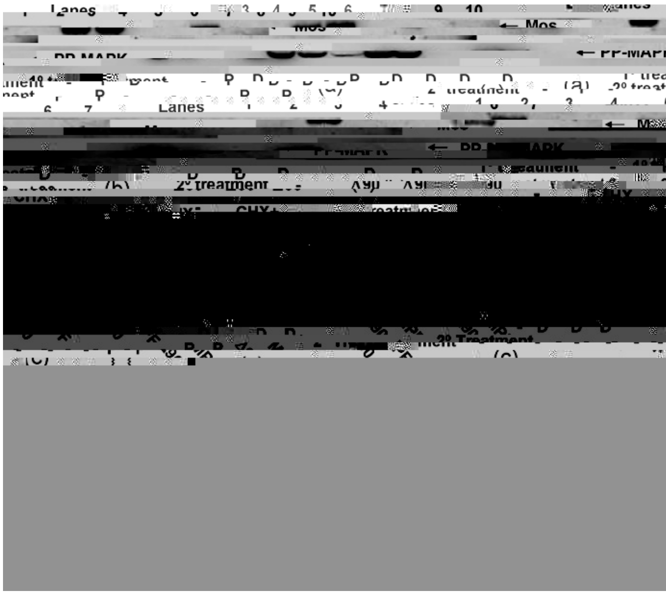


Fig. 7. Effects of transient exposure to 2,4-D on Mos and PP-MAPK expression. (a) Ooc

Author's personal copy

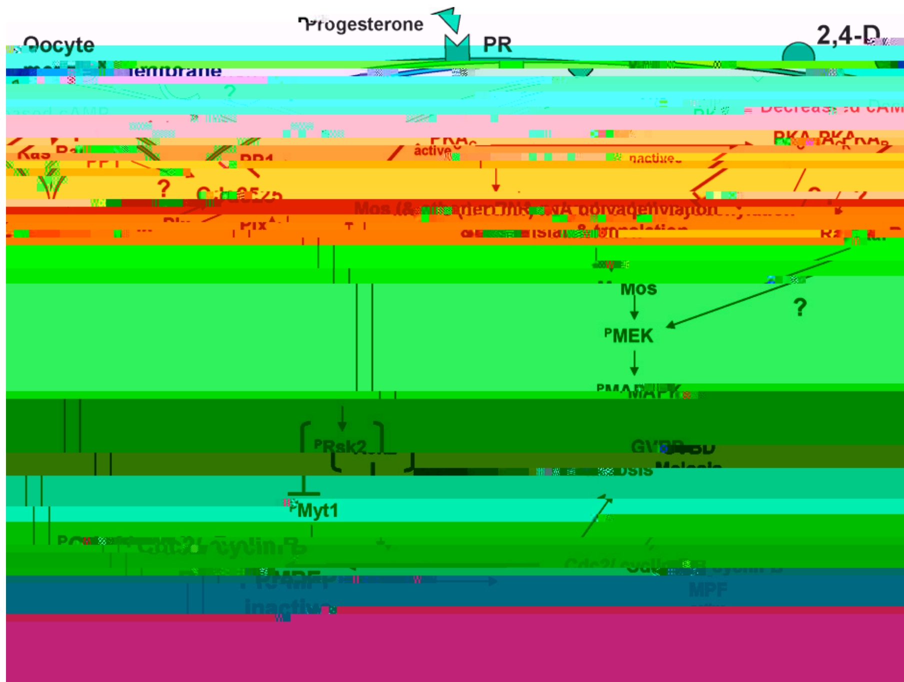


Fig. 8. Proposed model for maturation inhibition by 2,

Author's person

Author's personal copy

[58] Tung J, Hansen D, Ban K, Loktev A, Summers M, Adler III J, et al. A role for the anaphase-promoting complex inhibitor Emi2/XErp1, a homolog of early mitotic inhibitor 1, in cytostatic factor arrest of Xenopus eggs. Proc Natl Acad Sci USA 2005;102:4318–23.

[59] Hansen D, Tung J, Jackson P. CaMKII and polo-like kinase 1 sequentially phosphorylate the cytostatic factor Emi2/Xerp1 to trigger its destruction and meiotic exit. Proc Natl Acad Sci USA 2006;103:608–13.

Author's personal copy