LOCALISED MPF ACTIVATION AND MITOTIC PHOSPHORYLATION IN FERTILISED *XENOPUS* EGGS

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In amphibians such as *Xenopus laevis*, the initial polarity of the oocyte is established during oogenesis to produce animal and vegetal hemispheres with extensive structural and compositional differences. The animal-vegetal polarity of the egg is reinforced by "Surface Contraction Waves" (SCWs), occurring immediately prior to each cleavage division (Hara et al., 1980; Sawai, 1982). These are instrumental in concentrating germ plasm at the vegetal pole (Savage and Danilchik, 1993). We hypothesised that the SCWs, and other animal-vegetal asymmetries in egg organisation, may result from localised activation of maturation promoting factor (MPF) in the animal hemisphere, where the mitotic spindle is positioned. MPF is a complex of active Cdc2 kinase and cyclin B, and is a universal activator of mitosis that can activate autonomously in egg cytoplasm. Mitotic phosphorylation, provoked directly or independently by MPF activation, affects the organisation of the cell by regulating, for instance, microtubule dynamics, myosin activity, microtubule-severing activity and microtubule motor protein activity.
We have examined the spatial regulation of MPF activation in *Xenopus* eggs at first mitosis and assessed the distribution of mitotic phosphorylation using the monoclonal antibody MPM-2 (Davis et al., 1983). MPM-2 reactive proteins include spindle proteins, centrosomal proteins and microtubule binding proteins, as well the phosphatase Cdc25 (Kuang et al., 1994) which enhances MPF activation in a positive feedback loop.

A wave of MPF activation

We investigated the possibility that MPF activity is restricted locally (Iwao et al., 1993) and/or spreads across the egg (Masui, 1972).At first mitosis, Histone H1 kinase activity (a measure of MPF) was compared in animal and vegetal hemispheres (fig. 1). MPF peaked first in the animal half of the egg at the time of mitotic metaphase, then subsequently in the vegetal half after a 5-10 minute delay (fig. 2 top). The post-mitotic fall in histone H1 kinase activity in the vegetal half was slower than in the animal half. To test whether MPF activation in the vegetal half was dependent on prior activation in the animal hemisphere, we separated living eggs into animal and vegetal fragments with glass rods well before mitosis (before 0.6 NT) and followed the subsequent kinetics of H1 kinase activation (fig. 2 botton). H1 kinase activity was found to increase in both animal and vegetal halves. MPF in the vegetal fragments can thus activate independently, albeit more slowly than in animal fragments, with propagated autocatalytic MPF activation in the cytoplasm playing little or no role in vegetal MPF activation at first mitosis.

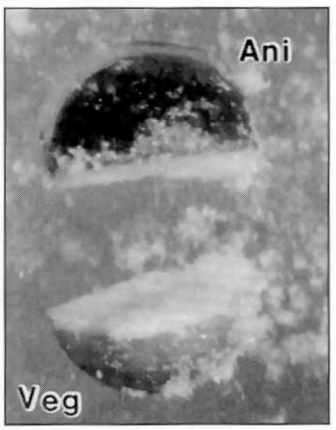

Figure 1. Fertilised *Xenopus* egg (approx. 1.2 mm diameter) frozen in liquid nitrogen and bisected along the equatorial pigment boundary.

Localised mitotic phosphorylation

To examine the localisation of phosphorylation resulting from MPF activation, we used the anti-mitotic phosphoprotein antibody MPM-2 on western blots.
Phosphorylation changes of some proteins accompanying transitory MPF activation at first mitosis, were restricted to the animal hemisphere. Most reactive phosphoproteins were represented similarly in both halves of the egg, however, some were localised animally or vegetally. Reactive bands were quantitatively higher in the animal hemisphere. MPM-2 reactive bands on western blots were detectable during both interphase and M phase of the *Xenopus* first cell cycle. The intensity of some MPM-2-reactive bands increased slightly during the period of mitosis (0.7-0.9 NT). These changes in mitotic phosphorylation were clearly detectable in animal but not in vegetal hemispheres . In some cases this resulted in the level of MPM-2 reactivity being greater in the vegetal hemisphere at the time of cleavage. The persistant 'mitotic' phosphorylation in the vegetal hemisphere partially explains the high level of MPM-2 phosphoproteins detectable throughout the first cell cycle in whole eggs.

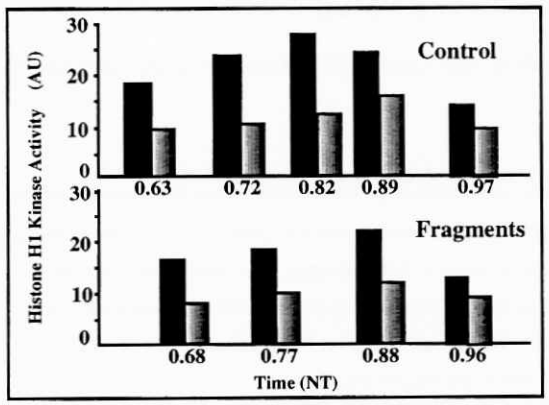

Figure 2. Histone H1 kinase activity in animal and vegetal hemispheres and fragments. H1 kinase activity from groups of 3 animal (black bars) or vegetal (grey bars) fragments was measured for each time point. Activity in samples is expressed in arbitrary units derived from scintillation counts and standardised with respect to the protein concentration in the assayed samples. In "Normalised Time (NT) 0 is the time of Insemination and 1. 0 the time of first cleavage.

Mechanism of localised MPF activation

Two possible regulatory mechanisms might account for the regionalised MPF activation, preferential cyclin B accumulation or localised Cdc2 dephosphorylation at tyrosine 14/ threonine 15. To address these possibilities we followed the behaviour of Cdc2 and cyclin B in animal and vegetal halves by western blotting (Fig. 3). Cdc2 was found to be distributed evenly throughout the egg, while Eg2, another *Xenopus* egg kinase, which associates with spindle poles, was strongly enriched in animal cytoplasm.The anti -Cdc2 antibody we used could detect the appearance of the two slower migrating forms that have been shown to correspond to the small population of inactive, tyrosine 14 and/or threonine 15-phosphorylated Cdc2 (Solomon et al., 1990; Ferrel et al., 1991). A clear lag in Cdc2 dephosphorylation between hemispheres corresponded closely with the pattern of MPF activation. Cyclin B2 was found to accumulate at similar rates in animal and vegetal cytoplasm

(Fig. 3). In addition, cyclin B2 destruction at the end of mitosis appeared to be more complete in the animal half and delayed and less complete in vegetal half, accounting for the observed difference in MPF inactivation. It thus appears that the advanced MPF activation in animal cytoplasm is more likely to result from enhanced activating dephosphorylation of Cdc2 than from localised cyclin B accumulation. We think that the nucleus or spindle may have an enhancing role in MPF activation (Ookata et al., 1992; Iwao et al., 1993) by facilitating Cdc2 tyrosine dephosphorylation.

Relationships between MPF and SCWs

The timing and direction of the first SCW coincides with the wave of MPF activation passing from animal to vegetal poles, and the second SCW coincides with the wave of MPF inactivation (see Fig. 4). It seems, however, that the shift in MPF activity may not provoke SCWs directly. We found that MPF activation can occur autonomously in vegetal egg fragments (fig. 2), whereas *in vivo* separation has been shown to block the rounding up accompanying the second SCW (Shinagawa, 1985), as well as the animal-vegetal progression of the cleavage furrow (Kubota and Sakamoto, 1993). Either the level of MPF activation in vegetal fragments is insufficient to trigger a response, or MPF targets localised in the animal hemisphere initiate a cascade of other kinases and/or structural modifications (Shinagawa ,1985).

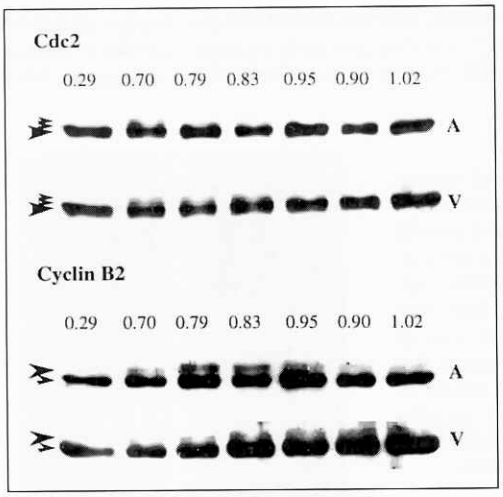

Figure 3. (left) Differential behaviour of Cdc2 and cyclin B in animal and vegetal hemispheres of whole eggs frozen at intervals during the first cell cycle. Times of egg freezing (NT) are shown. Western blot of a 12% polyacrylamide gel probed with anti-Cdc2 monoclonal (#17, Santa Cruz Biotechnology) and anti *Xenopus* cyclin B2 rabbit polyclonal serum (gift of M. Dorée). Smaller arrowheads in the Cdc2 blot correspond to the inactive forms. In the Cyclin B2 blot, large arrows:cyclin B, small arrows: non-specific band.

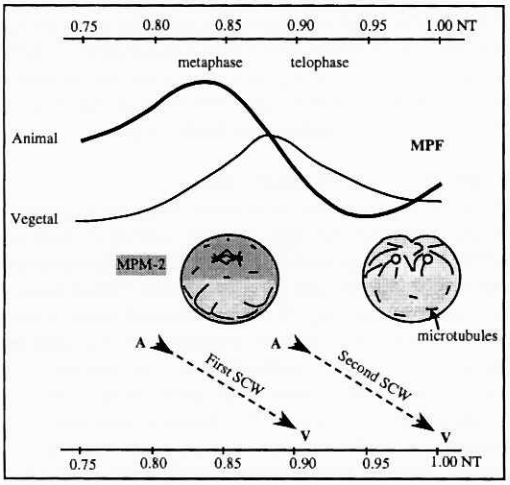

Figure 4. Schematic diagram indicating how the animal-vegetal shift of MPF (Histone H1 kinase) activity relates to changes in mitotic phosphorylation detected with MPM-2, microtubule reorganisation and Surface Contraction Waves (SCWs) passing from animal to vegetal. The timing of SCWs and mitotic events is taken from Ubbels et al. (1983).

CONCLUSIONS

1. MPF activation at first mitosis in the vegetal hemisphere is delayed with respect to the animal hemisphere.
2. Propagated autocatalytic MPF activation plays little or no role in the spatial MPF wave at first mitosis.
3. Phosphorylation of some of the MPM-2 reactive proteins is restricted to the animal hemisphere at first mitosis.
4. MPF activation in animal cytoplasm seems more likely to result from enhanced activating dephosphorylation of Cdc2 than from localised cyclin B accumulation.
5. MPF inactivation in both halves follows the same kinetics as cyclin B2 destruction, with Cyclin B2 destruction more complete in the animal hemisphere.

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