Expression of cell-cycle regulators during *Xenopus* oogenesis

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keywords: Xenopus; oogenesis; cell-cycle regulator

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Word count: 963 words (including Abstract and Text, but excluding References and Figure Legends).

Abstract

In full-grown *Xenopus* oocytes, cell-cycle regulators and pre-MPF are stored ready to bring about a specific cell cycle for oocyte maturation. We examined the expression pattern of these cell-cycle regulators as well as pre-MPF formation during oogenesis. Cdc2 and Cyclin B2 were already present in stage I oocytes and pre-MPF formation was also detected in stage I oocytes. Some negative regulators of MPF, Myt1 and Chk1, were synthesized early in oogenesis. In contrast, positive regulators of MPF, MEK, MAPK and Cdc25C, were mainly synthesized late in oogenesis. Northern blotting analysis suggested that the synthesis of these cell-cycle regulators was controlled by translation.

1. Results and discussion

Oogenesis is a continuous, asynchronous process and oocytes grow gradually over a period of eight months (Smith et al., 1991). During this period, the expression of cell-cycle regulators may be regulated to bring about a specific cell cycle for oocyte maturation. In fact, an inactive form of maturation/M phase promoting factor (pre-MPF) is stored in oocytes (Gautier and Maller, 1991; Kobayashi et al., 1991). Therefore, we investigated the expression of Cyclins and Cdks as well as other cell-cycle regulators during *Xenopus* oogenesis.

1.1. Expression of Cdc2 and Cyclin B2, and pre-MPF formation during Xenopus oogenesis

Cyclin B2 and Cdc2 were detectable in the stage I oocytes and their amounts began to increase greatly at stage IV (Fig. 1A). In addition, phosphorylation of Cdc2 on Tyr15 was also clearly detected in stage I oocytes (Fig. 1B). This result indicates that pre-MPF is already present at oogenesis even earlier than previously reported (Tayor and Smith, 1987).

The levels of mRNA were analyzed by Northern blotting. The amount of Cyclin B2 mRNA and Cdc2 mRNA was increased and reached a plateau at stage III (~20 pg/oocyte and ~7 pg/oocyte,

respectively) (Fig. 1C).

1.2. Expression of negative regulators for oocyte maturation during Xenopus oogenesis

Wee1 gradually decreased in amount and disappeared at stage VI, as reported previously (Nakajo et al., 2000). In sharp contrast, Myt1 was already present in stage I oocytes. It then showed a dramatic increase in quantity until stage IV. Chk1 was faintly detected in oocytes of stage I, and its amount increased markedly until stage V. The band of Cds1 was also faint in oocytes of stage I, but remained constant until stage IV. The intensity of this band was greatly increased after stage V.

Northern blotting showed that Wee1 mRNA slightly increased in quantity until stage III (~20 pg/oocyte), after which it remained roughly constant (Fig. 2B). The Myt1 and Chk1 mRNA detected in stage I oocytes reached a peak at stage III (~one pg/oocyte and ~4 pg/oocyte, respectively) and then decreased. Cds1 mRNA detected in stage I oocytes increased in quantity until stage III (~3 pg/oocyte), then this band became broader and shifted faster after stage IV.

1.3. Expression of the positive regulators of MPF during Xenopus oogenesis

MEK was detected in stage I oocytes, and showed a consistent increase in quantity throughout oogenesis (Fig. 3A). MAPK and Cdc25C were also detected in stage I oocytes, but their quantity remained roughly constant until stage III, although the level of Cdc25C in stage I oocytes was actually very low. After stage IV, they both increased in quantity.

As shown in Fig. 3B, the mRNA of each of the positive regulators examined also increased in quantity until stages III-IV (in the case of MEK and MAPK, ~5 pg/oocyte and in case of Cdc25C, ~7 pg/oocyte) and then decreased.

1.4. Expression of other cell-cycle regulators during Xenopus oogenesis

Cyclin B1 was first detected in stage V oocytes but did not increase in quantity until stage VI (Fig. 4A). Nek2B was detected faintly in stage I oocytes but did not increase in quantity until stage IV, after which the amount dramatically increased. In contrast, Cdk2 was detected in stage I oocytes and its amount increased steadily until stage VI.

Northern blotting analysis showed that the amount of Cyclin B1 mRNA increased slightly until stage III (~20 pg/oocyte), after which it remained constant. The levels of Nek2B mRNA and Cdk2

mRNA increased, reaching a peak at stage III (~4 pg/oocyte and ~3 pg/oocyte, respectively), after which they decreased in quantity.

In summary, pre-MPF is already present in stage I oocytes. Some of the negative regulators of MPF increase in quantity dramatically at an early stage of oogenesis. Interestingly, the synthesis of these regulators precedes both the synthesis of positive regulators and pre-MPF formation. The amount of many kinds of mRNA examined peaked at stages III-IV, before decreasing. This decrease may reflect the slight degradation of these mRNAs during oogenesis. The profile of the expression of these proteins is different from that of their mRNA, suggesting the synthesis of proteins examined may be regulated at translation, either negatively or positively.

2. Experimental procedures

Ovaries were excised from *Xenopus* females and treated with collagenase as described (Furuno et al., 1994). Staging of the oocytes was done according to Dumont (1972).

Oocyte extracts were prepared in EB buffer (Gerhart et al., 1984). The amount of the proteins derived from each stage of oocytes was quantified by dot blot and SDS gel electrophoresis,

followed by staining with Coomassie brilliant blue. Oocyte extracts, equivalent to one half of a stage VI oocyte, were subjected to Western blot analysis.

RNA was extracted from oocytes of various stages using Trizol reagent (GIBCO BRL). mRNAs used for quantification were synthesized *in vitro* by using MEGAscript (Ambion). Northern hybridization was performed as described (Furuno et al., 1988). Quantification of each mRNA was performed by a Fuji BAS2000 imaging analyzer.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan. The English used in this manuscript was revised by Miss K. Miller (Royal English Language Centre, Fukuoka, Japan).

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Figure legends

Figure 1. Expression of Cyclin B2 and Cdc2, and pre-MPF formation during oogenesis. (A) Western blotting analysis of Cyclin B2 and Cdc2. Oocyte extracts prepared from the smallest stage I oocytes to stage VI oocytes were subjected to Western blotting analysis with anti-PSTAIR (Furuno et al., 1994) or anti-Cyclin B2 antibody (Yoshitome et al., 1998), respectively. Routinely, oocyte extracts equivalent to one half of a stage VI oocyte were used in this assay. I, II, III, IV, V and VI indicate stages I, II, III, IV, V and VI, respectively. Since the size of each stage varied widely, we chose the oocytes according to the following criteria: oocytes of stages I, II, III, IV, V and VI were about 50 μm in diameter and transparent, about 300 μm and translucent, about 500 μm and light brown, of minimum size with differentiated animal and vegetal hemispheres, about 1,000 μm without a white band and 1,200 μm with a white band, respectively. Cyclin B2 was detected in two forms, as previously reported in stage VI oocytes. The band indicated by a filled triangle corresponds with the phosphorylated form of Cdc2 on Thr14 and Tyr15, whose residues become phosphorylated when Cdc2 associates with Cyclin B (Solomon et al., 1990). (B) Pre-MPF formation during oogenesis. Extracts of oocytes from each stage were subjected to Western blotting with Anti-phospho-Tyr (Cdc2) (purchased from Cell

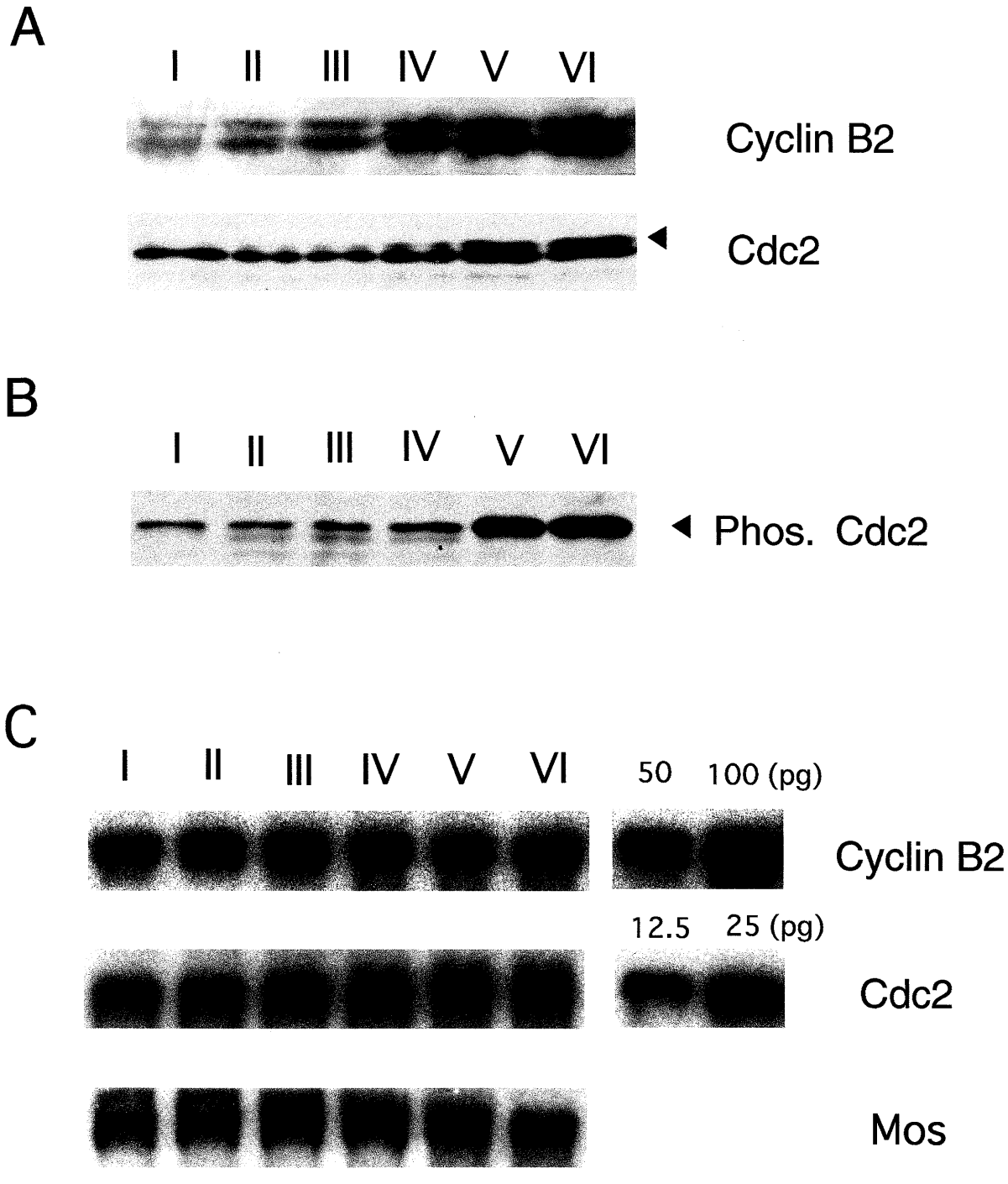
Signal Technology). The phosphorylated form of Cdc2 is indicated by a filled triangle. (C) Northern blotting analysis of Cyclin B2 and Cdc2. Total RNA was extracted from 100 oocytes of stages I and II, 20 oocytes of stage III and 10 oocytes of stages IV to VI. Total RNA equivalent to that of two and a half oocytes prepared from oocytes of each stage was subjected to Northern blotting using Cdc2 or Cyclin B2 cDNA as the probe. Usually, total RNA of the same number of oocytes of the same stages is used for Northern blotting analysis or RNase protection assays (Sagata et al., 1988; Kobayashi et al., 1991; Paris et al., 1991), because the amount of rRNA is greatly increased during oogenesis. Mos mRNA was used for the loading control. The amount of this mRNA is reported to remain fairly constant during oogenesis (Sagata et al., 1988). Pure Cyclin B2 or Cdc2 mRNA transcribed *in vitro* was used as a standard for estimating the mRNA concentration.

Figure 2. Expression of negative regulators for the initiation of oocyte maturation during oogenesis. (A) Western blot analysis of Wee1, Myt1, Chk1 and Cds1. Western blotting was performed with anti-XeWee1 (Nakajo et al., 2000), anti-XeMyt1 (Nakajo et al., 2000), anti-XeChk1 (Nakajo et al., 1999) or anti-XeCds1 antibody (Gotoh et al., 2001), respectively. (B) Northern blotting analysis of Wee1, Myt1, Chk1 and Cds1. Total RNA was extracted from oocytes of each stage and the mRNA of Wee1, Myt1, Chk1 and

Cds1 was detected by using each of the cDNAs as the probe. Pure Wee1, Myt1, Chk1 or Cds1 mRNA made *in vitro* was used as a standard for estimating the mRNA concentration.

Figure 3. Expression of positive regulators of MPF during oogenesis. (A) Western blotting analysis of Cdc25C, MEK and MAPK. Western blotting was carried out with anti-XeCdc25C (Nakajo et al., 1999), MEK (purchased from Santa Cruz) or MAPK antibody (purchased from Santa Cruz), respectively. (B) Northern blotting analysis of Cdc25C, MEK and MAPK. The mRNA of Cdc25C, MEK and MAPK was detected by using Cdc25C, MEK or MAPK cDNA as the probe. Pure MEK, MAPK or Cdc25C mRNA transcribed *in vitro* was used as a standard for estimating the mRNA concentration.

Figure 4. Expression of Cdk2, Nek2B and Cyclin B1 during oogenesis. (A) Western blotting analysis of Cdk2, Nek2B and Cyclin B1. Western blotting was performed with anti-Cyclin B1 (Yoshitome et al., 1998), Nek2B (Uto et al., 1999) or Cdk2 antibody (Furuno et al., 1997), respectively. (B) Northern blotting analysis of Cdk2, Nek2B and Cyclin B1. Total RNA extraction and Northern blotting were performed as described in Fig. 1. Each mRNA was probed by using Cyclin B1, Nek2B or Cdk2 cDNA. Pure Cyclin B1, Nek2B or Cdk2 mRNA made *in vitro* was used as a standard for estimating the mRNA concentration.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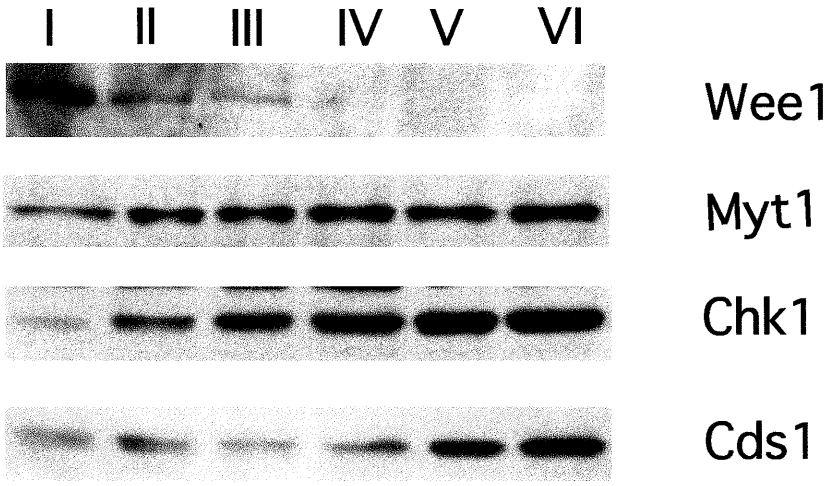
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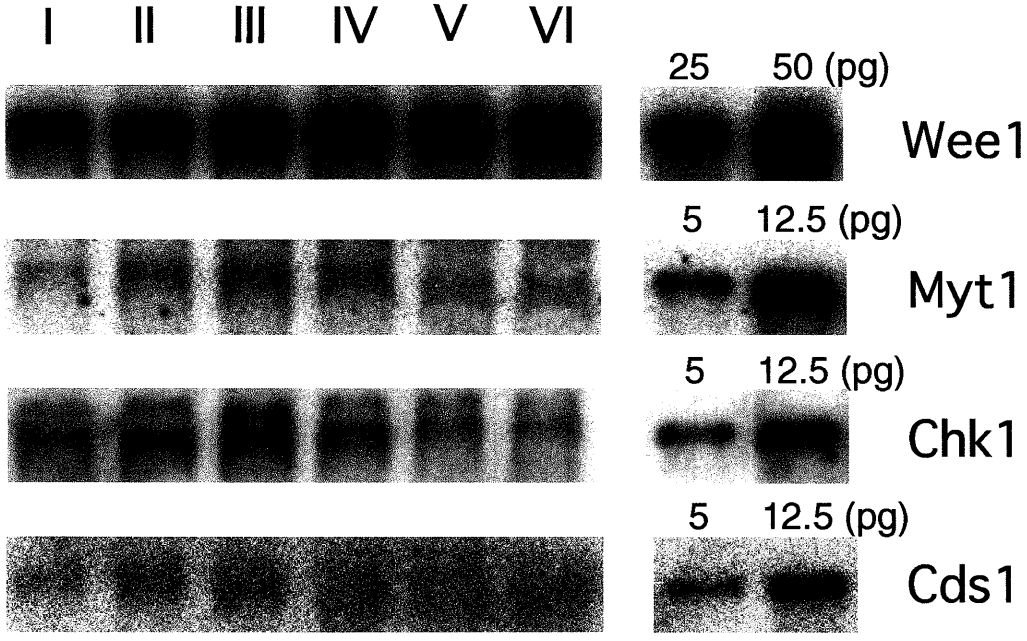
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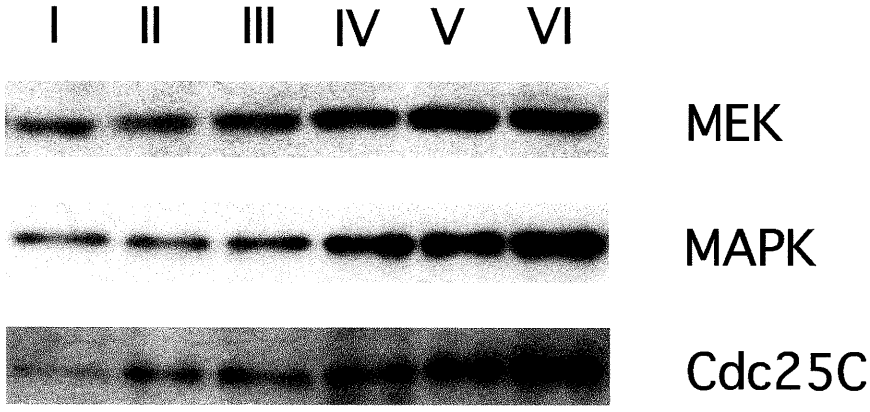
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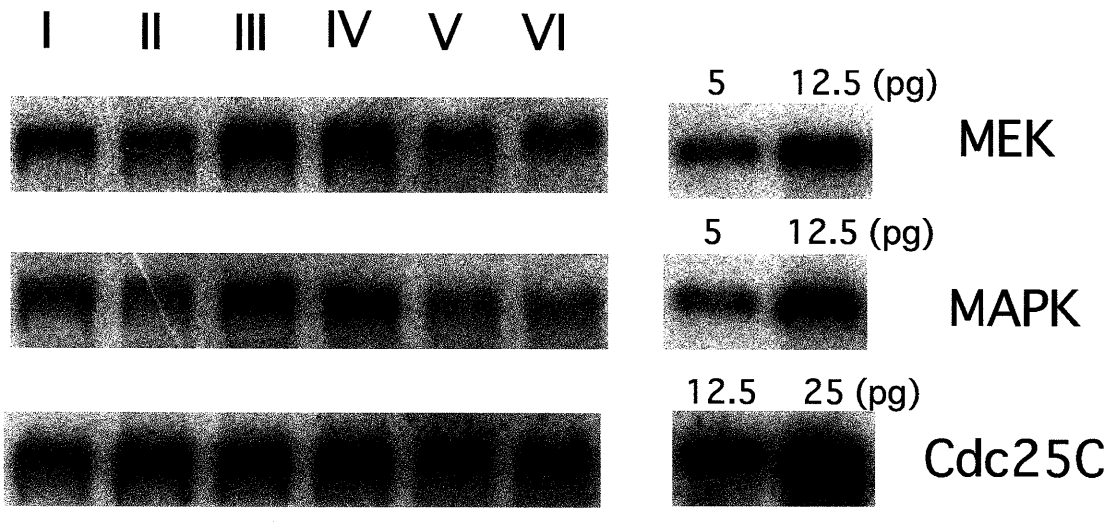
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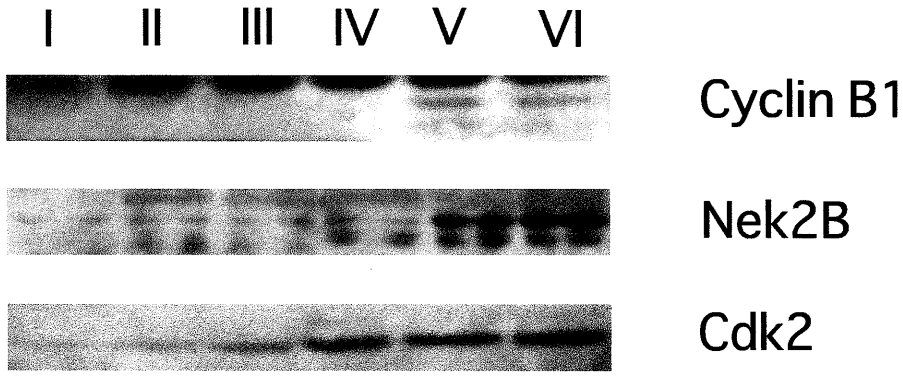
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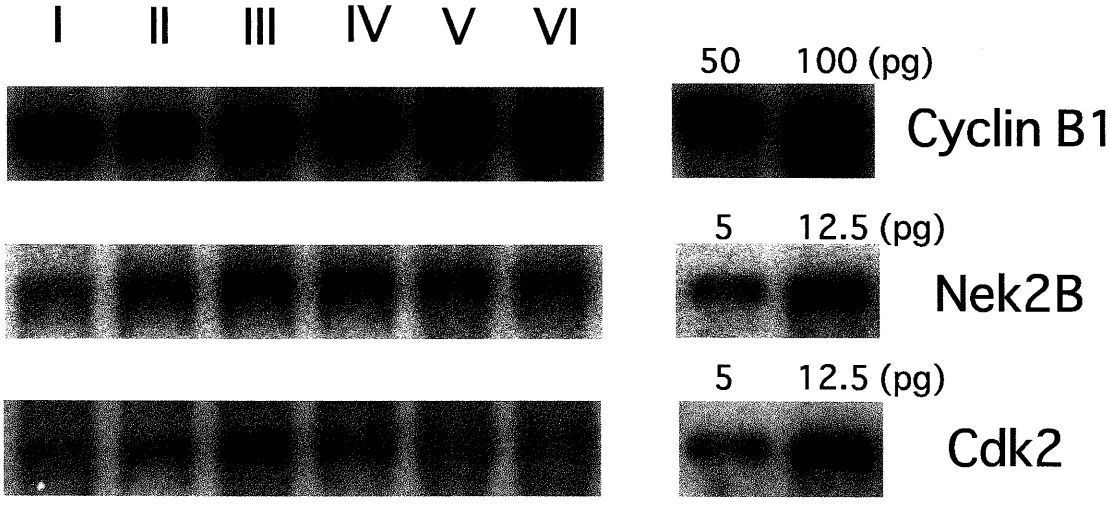
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