抗体注射に関する論文

題名：Involvement of the protein of Xenopus vasa homolog (Xenopus vasa-like gene 1,XVLG1)in the differentiation of primordial germ cells

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Materials and Methods

Microinjection

Microinjection into 32-cell embryos that were placed upside down in sterile Steinberg’s saline with 5% Ficoll (Type 400, Sigma) was carried out essentially int the same manner as described previously(Ikenishi et al.1986).About 2 nL of the above-mentioned antibodies with, or Steinberg’ saline with FDL, was injected via a micropipette (10 μm in outer tip diameter) into single vegetal blastomeres with a ‘dark area’ (Ikenishi & Nakazono 1986 ) or those containing the germ plasm of the embryos (Fig.1). Antibodies were injected at a final concentration of 0.8, 2 or 4mg/mL .Only embryos that cleaved normally after the injection and had no exudate at stage 9(late blastula) were transferred to full strength Holtfreter’s solution (pH7.2) and kept at 22℃ for another 12h.They were then transferred to sterile tap water and reared to the stages examined.

5%フィコールを加えた滅菌スタインバーグ生理食塩水（400型、シグマ）に逆さに入れた32細胞胚へのマイクロインジェクションは、基本的に以前（Ikenishi et al.1986）と同じ方法で行われた。 池西・中園 1986），または胚の生殖質を含む胚盤胞に，マイクロピペット（先端外径 10μm）を用いて上記抗体，または FDL を含む Steinberg' Saline を「dark area」の単一の植物極側に約 2nL注入した（図 1）。抗体は 0.8, 2, 4mg/mL の最終濃度で注入した。注入後正常に裂開し，第9期（後期胞胚）に滲出物がない胚のみを全濃度ホルトフレーター溶液（pH7.2）に移し，22℃でさらに12時間保持し，無菌水道水に移して調べたステージまで飼育した。

The FDL at a final concentration of 50-100mg/ml in Steinberg’ saline was injected with 2L-13 antibody

FDLをSteinberg'生理食塩水に50-100mg/mlの最終濃度で溶解し、2L-13抗体とともに注入した

FDL dissolved in modified Steinberg’s saline was sterilized with membrane filter and kept at

Differentiation of FDL-injected blastomeres

About 2nl of FDL at a concentration of 50,100 or 200 mg/ml in modified Steinberg’ saline was injected into signal blastomeres containing the germ plasm of Xenopus 32-cell embryos. A concentration of 50-100 mg/ml FDL was found to be sufficient for tracing the descendant cells from the injected blastomeres at the feeding tadpole stage. There were no significant differences in the number of FDL-labeled PGC at the tadpole stage, irrespective of the concentration of FDL, and the labeled somatic cells as well as the labeled PGC were easily identified at this concentration.

FDLを注入した胚珠の分化過程

Xenopus 32細胞胚の生殖質を含むシグナルブラストメアに，50，100または200 mg/mlの濃度のFDLを2nlほど注入した。50-100 mg/mlのFDL濃度は、注入した胚盤胞から摂食期の子孫細胞を追跡するのに十分であることが分かった。オタマジャクシ期におけるFDL標識PGCの数にはFDLの濃度に関わらず有意差はなく、この濃度では標識PGCだけでなく標識体細胞も容易に同定できた。