fere with its interaction with β-arrestin prevents this receptor from becoming internalized by the cell and instead triggers activation of p42/p44 MAPK through a separate route, resulting in translocation of p42/p44 MAPK to the nucleus and mitogenic stimulation of the cell (*7*).

Several findings now converge to enlarge the part played by arrestins in signal termination. Receptor signaling and desensitization of the receptor to the activating signal are in reality two intimately coupled processes. Molecules viewed as "signal terminators" in one pathway may in fact be "activators" in another. With the McDonald *et al.* work, we are witnessing an important new beginning for arrestins as activators of MAPK signaling.

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PERSPECTIVES: GENETICS

Reprogramming X Inactivation

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Several mammalian species including mouse (*1–3*), sheep, and pig have been successfully cloned by somatic cell nuclear transfer—that is, transfer of a nucleus from a differentiated adult cell into an oocyte that has had its own nucleus removed. Yet, despite these successes, few cloned embryos develop to term. Although the precise reasons for this are not clear, it is possible that the adult nucleus cannot be easily reprogrammed to direct embryonic development. On page 1578 of this issue, Eggan and colleagues (*4*) investigate how the transplanted nuclei of adult somatic cells and of embryonic stem cells are reprogrammed in cloned female mouse embryos. By monitoring X chromosome gene expression in different embryonic and extraembryonic (placental) tissues, the investigators were able to examine reprogramming of the X chromosome inactivation state of adult nuclei.

One of the two X chromosomes in female cells has to be shut down (transcriptionally silenced), otherwise twice the amount of protein will be produced in female cells as in male cells (which have only one X chromosome). There are several molecular processes that contribute to X chromosome inactivation [for a review, see (*5*)]. These include coating of the X chromosome by *Xist* nuclear RNA, removal of acetyl groups (hypoacetylation) from the histone proteins of DNA, accumulation of the macroH2A histone protein, and addition of methyl groups to CpG islands (stretches of cytosine and guanine nucleotides in the DNA). During the earliest phases of female embryogenesis, there is gene expression from both the maternal X chromosome (donated by the egg) and the paternal X chromosome donated by the sperm (see the figure, top). At the blastocyst stage, the X chromosome inherited from the father is preferentially chosen for inactivation in cells of the trophectoderm (the blastocyst outer layer). Later, when the embryo becomes implanted in the uterine wall, random X inactivation occurs in cells of the inner cell mass that will form all of the somatic tissues (*5*). Early inactivation events are under the control of a master region on the X chromosome, the Xic, which contains the *Xist* gene encoding a large nontranscribed nuclear RNA. Initiation of X inactivation involves both a step in which X chromosomes are counted relative to the total number of chromosomes in the cell and a process of choosing, whereby one of the two X chromosomes in the female cell may be preferentially selected for inactivation.

Eggan *et al.* studied X inactivation in cloned mouse embryos derived from enucleated oocytes injected with female adult cell nuclei (see the figure, bottom). In one set of experiments, the female adult nuclei transferred to oocytes had one X chromosome marked by a green fluorescent transgene (X^{GFP}) and the other marked by a null mutation in the *HPRT* gene. These cells are fluorescent when the transgene is carried by an active X chromosome (X_a) and is expressed, but are nonfluorescent when the transgene is carried by an inactivated X chromosome (X_i) and remains silent (*6*). Preparations of cells bearing an inactive X^{GFP} transgene ($X^{GFP}{}_i$) were obtained by selection with 6-thioguanine, which killed those cells expressing the normal *HPRT* gene; preparations of cells bearing an active X^{GFP} transgene ($X^{GFP}{}_a$) were obtained by selection in HAT medium, which killed those cells not expressing the *HPRT* gene. All blastocysts derived from [$X^{GFP}{}_i X_a$] nuclear transfer were fluorescent, supporting the conclusion that $X^{GFP}{}_i$ is reactivated in most cells of the preimplantation embryo. Reactivation of the X_i was followed by random inactivation of either X in the epiblastic cell lineages of cloned embryos (the epiblast, derived from the inner cell mass, will form all somatic tissues). This was known because transfer of either [$X^{GFP}{}_i X_a$] or [$X^{GFP}{}_a X_i$] adult nuclei resulted in a mixed population of cells bearing an $X^{GFP}{}_i$ and an $X^{GFP}{}_a$. Fluorescence-activated cell sorting of fibroblast cells derived from three mid-gestation E12.5 (embryonic day 12.5) clones revealed the $X^{GFP}{}_i$ and $X^{GFP}{}_a$ mixed cell populations. In extraembryonic tissues from four mid-gestation clones, the X inactivation status was determined by the donor cell nucleus because [$X^{GFP}{}_i X_a$] nuclei resulted in nonfluorescent placentas whereas [$X^{GFP}{}_a X_i$] nuclei generated fluorescent placentas.

The conclusions drawn from these elegant experiments were confirmed using polymorphisms (sequence variations) in the X-linked *Xist* and *Grpr* genes of embryos cloned from cells of F_1 females produced by crossing two mouse subspecies. Expression of *Xist* and *Grpr* from both X chromosomes (biallelic expression) confirmed that X reactivation followed by random inactivation had occurred in E13.5 tissues. In the placentas of six E13.5 cloned embryos, monoallelic expression of both *Xist* and *Grpr* was observed, which confirmed that X inactivation was nonrandom in extraembryonic tissues. Interestingly, transfer of nuclei from embryonic stem cells that have two active X chromosomes was associated with biallelic expression of *Xist* and *Grpr* in the placenta of the single recovered mid-gestation embryo. Thus, X inactivation in the extraembryonic territory of cloned embryos seems to occur randomly in the absence of the inactivation profile provided by the adult cell nucleus.

Given the extreme stability of X inactivation in adult tissues, it may seem remarkable that the early embryo can reverse the pattern of X inactivation (a process normally associated with formation of female gametes). It is possible, however, that the cloned embryo is mimicking properties of the natural embryo. During the early stages of normal embryogenesis, *Xist* RNA coats the paternal X chromosome (see the figure, top) (*7*). This

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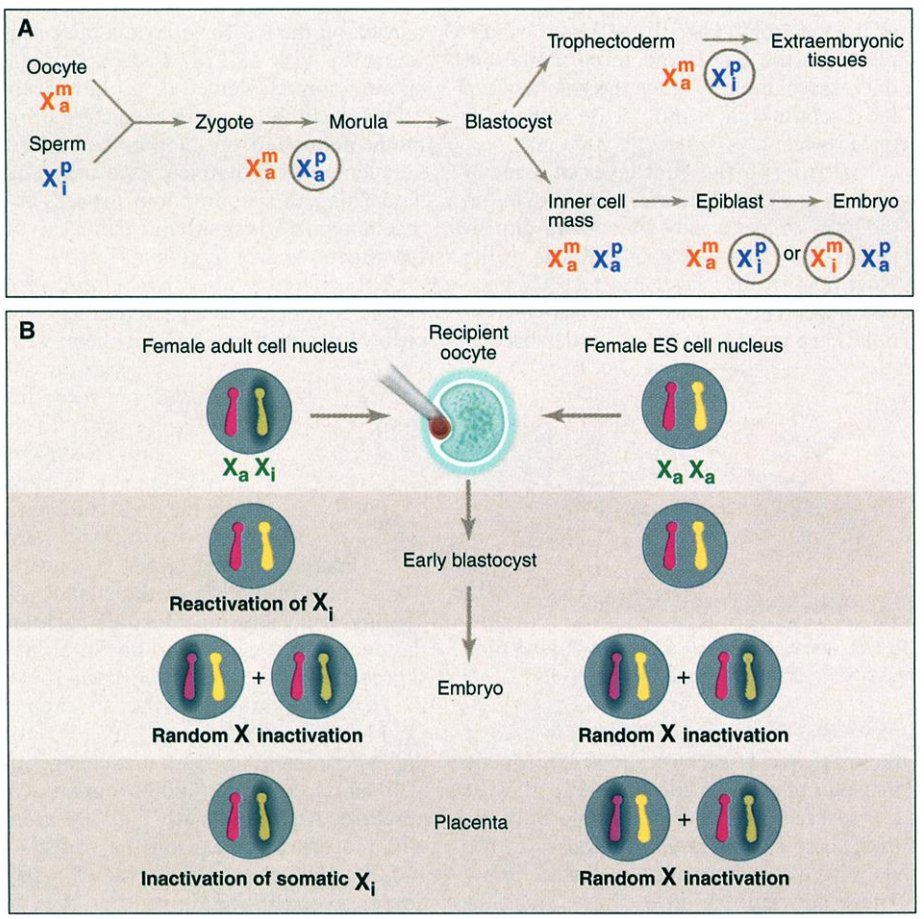
epigenetic mark disappears from the inner cell mass before the onset of random X inactivation. It is possible that a similar erasure of epigenetic constraints occurs on both the paternal X chromosome and the cloned X_i. However, the poor recovery of postimplantation embryos suggests that complete X_i erasure and reprogramming are inefficient. One-third of cloned embryos produced by nuclear transfer develop into blastocysts, but only a few percent make it through the critical period of implantation.

Eggan and colleagues suggest that there is a functional equivalence between the imprinted X after gamete formation and the somatic X_i in extraembryonic tissues. Methylation of the DNA could be involved because it is often suggested as an imprinting mark on the paternal X, and because CpG islands in the X_i are hypermethylated (*5*). Alternatively, the condensed state of X_i chromatin in the transplanted adult nucleus may be similar to that of the paternal X after gamete formation. Accumulation of histone macroH2A is associated with condensation of the paternal X in early embryos and extraembryonic cells of the blastocyst, and with condensation of the X_i in adult nuclei (*8*). A third possibility is the coating of the X chromosome by *Xist* nuclear RNA, which could be a mark both in the adult nucleus and in extraembryonic tissues. Indeed, it remains to be established whether the *Xist* coating is maintained or is removed from the paternal X and from the cloned X_i before imprinted inactivation is initiated in the trophectoderm. To explain the functional equivalence of the paternal X and X_i, one could also hypothesize that the X_a of the transferred adult nucleus is protected against inactivation in extraembryonic tissues of cloned embryos (comparable to the protection of the maternal X in extraembryonic tissues of the normal embryo). There is strong evidence that the maternal X normally carries a stringent imprint that confers resistance to inactivation in extraembryonic tissues. Female embryos that carry a deleted *Xist* gene on the maternal X inactivate the paternal X and grow normally; in contrast, those that have a paternal X with a deleted *Xist* gene die (*9*). The imprint must, at least in part, be carried by the region 3′ to the *Xist* gene, because maternal inheritance of a deletion including the *DXPas34* locus and abolishing *Tsix* antisense expression leads to aberrant maternal *Xist* expression and postimplantation embryonic lethality (*10*). It is unlikely that the imprint depends on methylation of this region, as it is not methylated in gametes or in preimplantation embryos (*11*).



X marks the spot. (**A**) X chromosomes in tissues of the developing female mouse embryo. X^m, maternal X chromosome; X^p, paternal X; X_i, inactive X; X_a, active X; circled X, X chromosome coated by *Xist* RNA. (**B**) X chromosomes in cloned female mouse embryos (*4*). Pink and yellow identify the individual X chromosomes; a blue shadow surrounds the inactive X. Transfer of an adult female somatic nucleus (X_aX_i) into an enucleated oocyte results in reactivation of X_i followed by random X inactivation in the embryo and inactivation of X_i in the placenta. Injection of an embryonic stem (ES) cell nucleus (X_aX_a) into an enucleated oocyte results in the random inactivation of both X chromosomes in embryonic and placental tissues.

The recovery of placentas that express genes from both X chromosomes after transfer of embryonic stem cell nuclei could signal that counting and random choice as well as imprinting occur in extraembryonic tissues. However, counterselection against X_aX_a cells and X_iX_i cells cannot be excluded. Androgenetic embryos that carry two copies of the paternal X also show random inactivation (*12*). Again, this may be due to secondary events, such as reactivation of one of the inactivated X chromosomes, rather than a counting process.

Exciting avenues of investigation are opened up by such nuclear transfer experiments. Many genes encoding factors that epigenetically modify chromosomal DNA can be mutated and tested with this technology. In combination with microarray analyses, it promises to reveal new molecules intervening in the X inactivation process. A fuller biochemical characterization of the X chromosomes of cloned embryos (including characterization of the *Xist* coating and histone acetylation) should elucidate the extent to which reprogramming of the inactive X chromosome in cloned cells recapitulates X inactivation during normal embryogenesis.

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