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STEM CELLS

A samurai without a master

> …a modern Ronin, which controls embryonic stem (ES)-cell pluripotency…

During the feudal period of Japan, a samurai without a master was called a ronin. Now, Dejosez and colleagues describe a modern Ronin, which controls embryonic stem (ES)-cell pluripotency and lacks any relationship to the known 'master' regulators of pluripotency (OCT4, SOX2 and NANOG).

Previous work showed that caspase-3, a component of the cell-death system, cleaves and depletes ES cells of NANOG, thereby inducing differentiation. Using caspase-3 as bait in a two-hybrid screen, the authors discovered Ronin, a nuclear protein with a zinc-finger DNA-binding domain, which is common in factors that are involved in the epigenetic silencing of gene expression.

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Using genetic deletion, the authors showed that Ronin is essential for embryogenesis and for the derivation and propagation of ES cells *in vitro*. They next demonstrated that the forced expression of Ronin allowed cells to proliferate without differentiation under conditions that do not support self-renewal.
So, Ronin maintains ES-cell pluripotency, but does it act together with the canonical pluripotency factors?

This does not seem to be the case, because knockdown of *Oct4*, *Sox2* and *Nanog* by small interfering RNA (siRNA) in ES cells did not affect Ronin expression. Furthermore, overexpression of Ronin in siRNA-treated cells could override the requirement of these factors in maintaining pluripotency. So, Ronin is indeed a 'samurai without a master', but how does it maintain pluripotency?

Gene expression profiling in Ronin-overexpressing ES cells revealed a broad transcriptional repression. The authors also detected a genome-wide increase in the dimethylation of Lys9 of histone H3 (H3K9me2; a repressive mark) in Ronin-overexpressing ES cells compared with wild-type cells. These findings suggest that Ronin functions as a transcriptional repressor.

Consistent with this hypothesis, Ronin bound to the promoters of *Gata4* and *Gata6* in undifferentiated ES cells that were also enriched in H3K9me2. Following differentiation, Ronin binding and the H3K9me2 mark were lost and the *Gata4* and *Gata6* genes were actively transcribed. Ronin also interacts with HCF1, a key transcriptional regulator, and is part of a large chromatin-modifying complex.

By showing that Ronin is a new type of pluripotency factor that functions through an epigenetic mechanism of gene repression, this study adds a new level of control to the OCT4, SOX2 and NANOG transcriptional circuit, which is believed to regulate stem-cell pluripotency. The authors propose that Ronin may act broadly on transcription in pluripotent cells, whereas the canonical pluripotent factors modulate specific genes that are required for either pluripotency or differentiation. These findings therefore suggest a need to reconsider the prevailing OCT4, SOX2 and NANOG-centric view of ES-cell pluripotency.

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ORIGINAL RESEARCH PAPER Dejosez, M. *et al.* Ronin is essential for embryogenesis and the pluripotency of mouse embryonic stem cells. *Cell* **133**, 1162–1174 (2008)