## Novel signalling in mouse embryonic stem cells alters the pluripotent state

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*(Introduced by Derek Laver)*

The phenotypic status of embryonic stem (ES) cells is controlled in part by signalling pathways which translate inputs by extracellular molecules. An important extracellular protagonist in mouse ES cells is LIF (leukaemia inhibitory factor) which interacts with the gp130/LIFR membrane-receptor complex to activate a number of downstream signalling arms, including the STAT3, MEK/ERK and PI3K/Akt pathways. These pathways, together with others, interact in complex and sometimes competing ways to generate the well-known phenotypic characteristics of mouse ES cells of self-renewal, high rates of proliferation, and pluripotence.

The addition of a second molecule, L-proline, to the extracellular environment alters the pluripotent status of mouse ES cells, converting them to a second pluripotent cell population equivalent to the primitive ectoderm of the pre-gastrulating embryo. This conversion, from ES cells to primitive ectoderm-like cells, primes the latter for directed differentiation to specific cell types (Bettess *et al.*, 2003). Here we show, using inhibitor studies and kinome array analysis, that this small molecule appears to work by i) changing the balance in activity of signalling pathways already stimulated by LIF, and ii) activating additional signalling pathways. Specifically, L-proline rapidly further activates the LIF-stimulated MEK/ERK pathway, tipping the balance in favour of differentiation and away from ES-cell self-renewal sustained by LIF-mediated activation of the STAT3 pathway. In addition, L-proline rapidly stimulates other pathways including p38, mTOR and PI3K/Akt each of which contributes, to a greater or lesser extent, to the conversion to primitive ectoderm-like cells.

These results indicate that through the addition of small, nontoxic activators and inhibitors of signalling pathways, the differentiation of pluripotent ES cells might be controlled sufficiently well for the homogeneous production of specific cell types suitable for use in animal models of human disease.

Bettess MD, Lonic A, Washington JM, Lake JA, Morris MB, Rathjen J & Rathjen PD. (2003) *Proceedings of the 1st Australian National Stem Cell Conference*, p229.