undifferentiated, illustrating the significance of species-specific differences, even among mammals. For example, the leukemia inhibitory factor (LIF)/Stat3 pathway, a key to mouse ES cell proliferation, does not support human ES cell proliferation and appears inactive in conditions that support human ES cells (Daheron L, *et al.*, Stem Cells 22:770-778 (2004); Humphrey R, *et al.*, Stem Cells 22:522-530 (2004); and Matsuda T, *et al.*, EMBO J. 18:4261-4269 (1999)).

[00010] Similarly, while bone morphogenetic proteins (BMPs) together with LIF support mouse ES cell self-renewal at clonal densities in serum-free medium (Ying Q, *et al.*, Cell 115:281-292 (2003)), they cause rapid human ES cell differentiation in conditions that would otherwise support self-renewal, such as culture on fibroblasts or in fibroblast-conditioned medium (Xu R, *et al.*, Nat. Biotechnol. 20:1261-1264 (2002)). Indeed, inhibition of BMP signaling in human ES cells is beneficial (Xu R, *et al.*, Nat. Methods 2:185-190 (2005)).

[00011] Still further, fibroblast growth factor (FGF) signaling is important to self-renewal of human ES cells, but apparently not for mice (Xu *et al.*, (2005), *supra*; and Xu C, *et al.*, Stem Cells 23:315-323 (2005)).

[00012] Accordingly, the art still seeks a set of potency-determining factors suited at least for use in methods for reprogramming primate (including human and non-human) somatic cells to yield pluripotent cells. Such cells, obtained without relying upon embryonic tissues, would be suited for use in applications already contemplated for existing, pluripotent, primate ES cells.

~~BRIEF~~ SUMMARY

[00013] The present disclosure~~invention~~ is broadly summarized as relating to methods for reprogramming differentiated, somatic, primate cells into pluripotent cells, and more specifically into iPS cells. As used herein, "iPS cells" refer to cells that are substantially genetically identical to their respective differentiated somatic cell of origin and display characteristics similar to higher potency cells, such as ES cells, as described herein. The cells can be obtained from various differentiated (*i.e.*, non-pluripotent and multipotent) somatic cells.

[00014] iPS cells exhibit morphological (*i.e.*, round shape, large nucleoli and scant cytoplasm) and growth properties (*i.e.*, doubling time; ES cells have a doubling time of about seventeen to eighteen hours) akin to ES cells. In addition, iPS cells express pluripotent cell-specific markers (*e.g.*, Oct-4, SSEA-3, SSEA-4, Tra-1-60, Tra-1-81, but not SSEA-1). iPS cells, however, are not immediately derived from embryos and can transiently or stably express one or

more copies of selected potency-determining factors at least until they become pluripotent. As used herein, "not immediately derived from embryos" means that the starting cell type for producing iPS cells is a non-pluripotent cell, such as a multipotent cell or terminally differentiated cell, such as somatic cells obtained from a post-natal individual.

[00015] In the methods described herein, at least two potency-determining factors can be introduced into, and expressed in, differentiated somatic cells, whereupon the somatic cells convert in culture to cells having properties characteristic of pluripotent cells, such as human ES cells (*i.e.*, express at least Oct-4, SSEA-3, SSEA-4, TRA-1-60 or TRA-1-81, but not SSEA-1, and appear as compact colonies having a high nucleus to cytoplasm ratio and prominent nucleolus), that can differentiate into cells characteristic of all three germ layers, and that contain the genetic complement of the somatic cells of a post-natal individual. Apart from genetic material introduced to encode the potency-determining factors, the reprogrammed (*i.e.*, converted) cells are substantially genetically identical to the somatic cells from which they were derived.

[00016] As used herein, a "potency-determining factor" refers to a factor, such as a gene or other nucleic acid, or a functional fragment thereof, as well as an encoded factor or functional fragment thereof, used to increase the potency of a somatic cell, so that it becomes pluripotent. The potency-determining factors optionally can be present only transiently in the reprogrammed cells or can be maintained in a transcriptionally active or inactive state in the genome of the reprogrammed cells. Likewise, the potency-determining factors can be present in more than one copy in the reprogrammed cells, where the potency-determining factor can be integrated in the cell's genome, can be extra-chromosomal or both. The potency-determining factors can include, but are not limited to, Stella (SEQ ID NO:1); POU5F1 (Oct-4; SEQ ID NO:2), Sox2 (SEQ ID NO:3), FoxD3, UTF1, Rex1, ZNF206, Sox15, Mybl2, Lin28 (SEQ ID NO:4), Nanog (SEQ ID NO:5), DPPA2, ESG1, Otx2 and subsets thereof. In some embodiments, as few as two potency-determining factors, *e.g.*, Oct-4 and Sox2, can be sufficient. Efficiency in obtaining reprogrammed cells, however, can be improved by including additional potency-determining factor, such as Lin28, Nanog or both.

[00016a] In one aspect, there is provided a method of reprogramming primate somatic cells, the method comprising the steps of: exposing a plurality of potency-determining factors to primate somatic cells obtained from a post-natal individual under conditions sufficient to reprogram the cells, wherein the potency-determining factors comprise Oct4, Sox2, and at least one of Lin28 and Nanog; and culturing the exposed cells to obtain pluripotent reprogrammed cells, wherein the reprogrammed cells are substantially genetically identical to the post-natal individual.

[00016b] In one aspect, there is provided a method reprogramming primate somatic cells, the method comprising the steps of: exposing a plurality of potency-determining factors to the primate somatic cells obtained from a post-natal individual under conditions sufficient to reprogram the cells, wherein the potency-determining factors comprise Oct4, Sox2 and at least one of Lin28 and Nanog; and culturing the exposed cells to obtain pluripotent reprogrammed cells, wherein the reprogrammed cells are substantially genetically identical to the post-natal individual.

[00016c] In one aspect, there is provided an enriched population of primate pluripotent cells produced according to a method comprising the step of: introducing a plurality of potency-determining factors into primate somatic cells obtained from a post-natal individual under conditions sufficient to express the potency-determining factors, thereby reprogramming the somatic cells to produce euploid primate pluripotent cells, wherein the potency-determining factors comprise Oct4, Sox2, and at least one of Lin28 and Nanog, and wherein the reprogrammed cells are substantially genetically identical to the post-natal individual.

[00016d] In one aspect, there is provided an enriched population of primate pluripotent cells produced according to a method comprising the step of: introducing a plurality of potency-determining factors into primate somatic cells under conditions sufficient to express the potency-determining factors, thereby reprogramming the somatic cells to produce euploid primate pluripotent cells, wherein the potency-determining factors comprise Oct4, Sox2, and at least one of Lin28 and Nanog.

[00017] In one embodiment~~a first aspect~~, the disclosure~~invention~~ relates to a replenishable, enriched population of pluripotent cells obtained from a post-natal individual, especially from a living individual, but optionally from a deceased individual. Cells in the enriched cell population express at least one

cell-type-specific marker, including, but not limited to, Oct-4, SSEA3, SSEA4, Tra-1-60, Tra-1-81 or combinations thereof and have other hallmarks of pluripotent cells, such as ES cells. In addition, the pluripotent cells may express alkaline phosphatase (ALP). Furthermore, the pluripotent cells may have a genome substantially genetically identical to that of a pre-existing, differentiated cell from the individual. Likewise, the pluripotent cells may have a genome that encodes at least one of the potency-determining factors, which may be transcriptionally active or inactive after reprogramming. Additionally, the potency-determining factors may be in a form of a reprogramming sequence in which a polynucleotide encoding the potency-determining factor is operably linked to a heterologous promoter. As used herein, "heterologous promoter" means a promoter that is operably linked to a polynucleotide for which the promoter does not normally initiate transcription.

[00018] In one embodiment~~a second aspect~~, the disclosure~~invention~~ relates to methods and compositions for identifying potency-determining factors required to reprogram somatic cells into pluripotent cells.

[00019] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure~~invention~~ belongs. Although suitable methods and materials for the practice or testing of the present disclosure~~invention~~ are described below, other methods and materials similar or equivalent to those described herein, which are well known in the art, can also be used.

[00020] Other objects, advantages and features of the present disclosure~~invention~~ will become apparent from the following specification taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[00021] FIG. 1 illustrates a site downstream from a human *Oct4* promoter into which a knock-in construct was introduced. In cells containing the knock-in construct, enhanced green fluorescent protein (EGFP) and neomycin phosphotransferase (NEO) are expressed when the *Oct4* promoter is active. These cells can be used to evaluate which factors can reprogram somatic cells into pluripotent cells.

[00022] FIGS. 2A-B illustrate human H1 ES cell differentiation. FIG. 2A shows schematics of myeloid precursor derivation and purification from human ES cells. FIG. 2B shows phenotypic analysis of differentiated cells obtained after Percoll® separation. Gray line: