

# Loss of desmoglein 2 suggests essential functions for early embryonic development and proliferation of embryonal stem cells

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**Desmoglein 2 (Dsg2) is a Ca<sup>2+</sup>-dependent adhesion molecule of desmosomes and is synthesized in all desmosome-bearing tissues from their earliest appearance onward. To examine the function of Dsg2, its gene was inactivated by homologous recombination in embryonal stem (ES) cells for the generation of knockout mice. DSG2<sup>-/-</sup> mice and a considerable number of DSG2<sup>+/-</sup> mice died at or shortly after implantation. On the other hand, DSG2<sup>-/-</sup> blastocysts developed an apparently normal trophoblast layer, the first tissue known to produce desmosomes, and hatched properly. Immunofluorescence analyses of these blastocysts showed, however, that the distribution of the desmosomal plaque protein desmoplakin was disturbed, whereas the adherens junction proteins E-cadherin and  $\beta$ -catenin appeared to be unaffected. Unexpectedly, we found that Dsg2 seems to be essential for the inner cell mass and the ES cell population derived therefrom. We present evidence that Dsg2, which is located in desmoplakin-negative wild-type ES cells in non-desmosomal junctions, is needed for normal ES cell proliferation. Our observations thus reveal that important Dsg2 functions are desmosome-independent during early development and are needed for ES cell and early embryo survival.**

## Introduction

Desmosomal adhesion is mediated by calcium-dependent cell adhesion glycoproteins of the desmocollin and Dsg types that interact laterally and transcellularly, and that recruit electron-dense cytoplasmic plaque material to facilitate the attachment of intermediate filaments (Trojanovsky and Leube, 1998; Kowalczyk et al., 1999). Both desmosomal cadherin types are encoded by a small multigene family, each of which consists of three cell type-restricted members (Koch et al., 1992; Trojanovsky and Leube, 1998; Kowalczyk et al., 1999). The clinical relevance of these cell adhesion molecules is revealed by the blistering diseases caused by autoantibodies against desmosomal cadherins, by the specific cleavage of Dsg1 by *Staphylococcus aureus* toxin, and by the genodermatosis striate palmoplantar keratoderma that is caused by DSG1 mutations (Stanley, 1993; Amagai et al., 2000; Hunt et al., 2001).

The importance of desmosomal cadherins for desmosome formation, cell adhesion, and intermediate filament anchorage has been documented in several studies. Thus, desmosomal plaque formation and intermediate filament anchorage are critically dependent on cytoplasmic desmosomal cadherin domains, which act as inducers and/or inhibitors of these functions depending on the specific molecular context and expression level (Trojanovsky et al., 1993, 1994a, b; Norvell and Green, 1998). In addition, dominant-negative desmosomal cadherin mutations prevent desmosome formation in cultured cells (Trojanovsky et al., 1993, 1994a; Norvell and Green, 1998; Hanakawa et al., 2000) and transgenic mice (Allen et al., 1996). Further experiments have demonstrated that antisense RNA interfering with the synthesis of desmosomal cadherins alters desmosomes (Roberts et al., 1998) and that blocking peptides corresponding to the cell adhesion region of desmosomal cadherins interfere with epithelial morphogenesis (Runswick et al., 2001). Ablation of DSG3 and the desmocollin 1 gene both result in increased epidermal fragility and various structural and functional skin abnormalities (Koch et al., 1997, 1998;

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Chidgey et al., 2001). On the other hand, the synthesis of desmosomal cadherins together with the plaque protein plakoglobin induces, under certain conditions, cell-cell adhesion in non-epithelial cells (Marcozzi et al., 1998).

Dsg and desmocollin isoforms 2 are the most abundant desmosomal cadherins, being also the first detectable desmosomal cadherins to be synthesized in the trophectoderm at the blastocyst stage (Collins and Fleming, 1995; Collins et al., 1995) and in all desmosome-containing tissues (Koch et al., 1992; Theis et al., 1993; Lorimer et al., 1994; Schäfer et al., 1994, 1996; King et al., 1997). Furthermore, their occurrence appears to be strictly linked to desmosomes with little mistargeting to non-desmosomal junctions or other membrane domains. Consequently, desmosomal cadherin-fluorescent protein chimeras have been used successfully to study the behavior of desmosomes in living cells (Windoffer et al., 2002). Here, we report the effects of germline inactivation of DSG2. We demonstrate that Dsg2 loss of function leads to embryonic lethality at the time of blastocyst implantation, although a normal-appearing trophectoderm is formed. We have also made the unexpected discovery that Dsg2 function is central to the normal progression of inner cell mass-derived ES cells, which do not express mature desmosomes.

## Materials and methods

### Generation of knockout mice

Partial mouse Dsg2 cDNA clone Dsg2.mc580 (Tian, 2000) was used to isolate a 17-kb DSG2 NotI-fragment from a 129/Sv-derived  $\lambda$ -Fix II library (Stratagene, La Jolla, CA, USA). To prepare a targeting vector, a 5.3-kb HindIII fragment containing exons 3–6 was subcloned together with a 2.5-kb KpnI/SphI intron 8 fragment into Bluescript KS derivative R3 (clone V4). Between these two fragments, the Sall fragment from pGTIRESp $\beta$ geopA (kindly provided by Dr. A. Smith; University of Edinburgh, Scotland, UK) was inserted as a combined selection and reporter cassette (targeting vector DSG2Bgeo; Fig. 1A). For targeting vector DSG2hyg, an internal ribosomal entry site together with a promoterless hygromycin phosphotransferase gene (from pIRES1hyg; Clontech Laboratories, Palo Alto, CA, USA) was inserted between the 5'- and 3'-homology fragments in V4.

NotI-linearized targeting vectors were electroporated (single pulse, 500 F, 0.24 kV) into ES cell strain R1 (kindly provided by Drs. Nagy and Rossant, University of Toronto, Canada) and selected for G418 or hygromycin resistance. In the latter case, embryonic feeder cells from strain C57BL/6J-TgN(pPWL512hyg)1Ems (Jackson Laboratory, Bar Harbor, Maine, USA) were used during selection with 250  $\mu$ g/ml hygromycin B. Knockout mice were produced from recombinant ES cell clones as described (cf. Eshkind and Leube, 1995). For Southern blot analyses, either an external SphI/HindIII fragment or an internal neo-probe was used. PCR primers d3 (5'-AGGAAATGAAGGGGC-TATT-3') and d13 (5'-CTCTGAAAGGCAAGTAAGT-3') were used to generate a 460-bp product from the wild-type allele, or primers d1 (5'-GCCTTCTGTAAAGTTGGGC-3') and d13 to generate a 585-bp fragment from the mutated allele. For genotyping of blastocysts, embryos were flushed out, rinsed several times with M2 medium (Sigma, St. Louis, MO, USA), lysed in 2  $\mu$ l medium plus 23  $\mu$ l deionized water, and incubated for 15 min at 95°C. Half of each sample was subjected to PCR amplification, and the products were analyzed by Southern blot hybridization with an end-labeled internal primer common to both amplification products. Transgene-induced  $\beta$ -galactosidase activity was determined (Hogan et al., 1994) by using fixation times ranging from 1 min for blastocysts and blastocyst outgrowths to 30 min for ear punches. After overnight staining at 37°C, cells were postfixed overnight at 4°C.

### Establishment of ES cell lines

Blastocysts were plated individually on mitomycin-C-treated murine embryonic fibroblasts in ES cell medium supplemented with 2000 U leukemia inhibitory factor (Life Technologies, Karlsruhe, Germany) per ml. Embryos hatched and attached to the feeder layer 2 d after plating. After an additional 4 d in culture, during which the inner cell mass grew out, cells were separated by using a drawn-out Pasteur pipette. Cells were transferred 4–5 d later into new wells, trypsinized and expanded. Genotyping was performed by Southern blot hybridization of DNA obtained from parallel samples grown without feeder cells.

### Microscopy

Uteri were fixed in 4% formaldehyde/PBS and embedded in paraffin. Serial sections were stained with hematoxylin. For whole-mount staining, E3.5 blastocysts were incubated in ES cell medium for 24 h, washed with PBS/2 mM MgCl<sub>2</sub>, and fixed in methanol (–20°C) for 8 min. Embryos were then treated with acetone (–20°C) for 1 min, washed with PBS/50 mM NH<sub>4</sub>Cl, and incubated in blocking buffer (1% BSA, 0.1% Triton X-100, 1% gelatine in PBS) for 3 h. After 5 min incubation in 0.4% Triton X-100 in PBS, embryos were washed (2  $\times$  5 min in PBS), incubated with primary antibodies overnight at room temperature and again with fresh antibodies for 1 h, washed (3  $\times$  20 min PBS) and incubated with secondary antibodies for 1 h. Finally, embryos were incubated in a drop containing DAPI for 5 min and washed (4  $\times$  10 min in PBS). Before mounting, embryos were washed once with H<sub>2</sub>O and then individually transferred onto slides. Standard immunofluorescence microscopy of ES cells was done as previously described (Strnad et al., 2001).

Primary antibodies were guinea pig polyclonal antibodies Mp6 reacting with human Dsg2 (Tian, 2000), monoclonal antibodies Dg3.10 against Dsg, DP 2.15/2.17/2.20 against desmoplakin, and PP2/86 against plakophilin 2 from Progen Biotechnics (Heidelberg, Germany), 11E4 against plakoglobin from Dr. M. Wheelock (University of Toledo, OH, USA), and monoclonal antibodies against E-cadherin and  $\beta$ -catenin from BD Transduction Laboratories (Franklin Lakes, NJ, USA). Cy2- and Cy3-conjugated secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, PA, USA).

Electron microscopy and immunoelectron microscopy (silver amplification technique) were performed as described (Strnad et al., 2001).

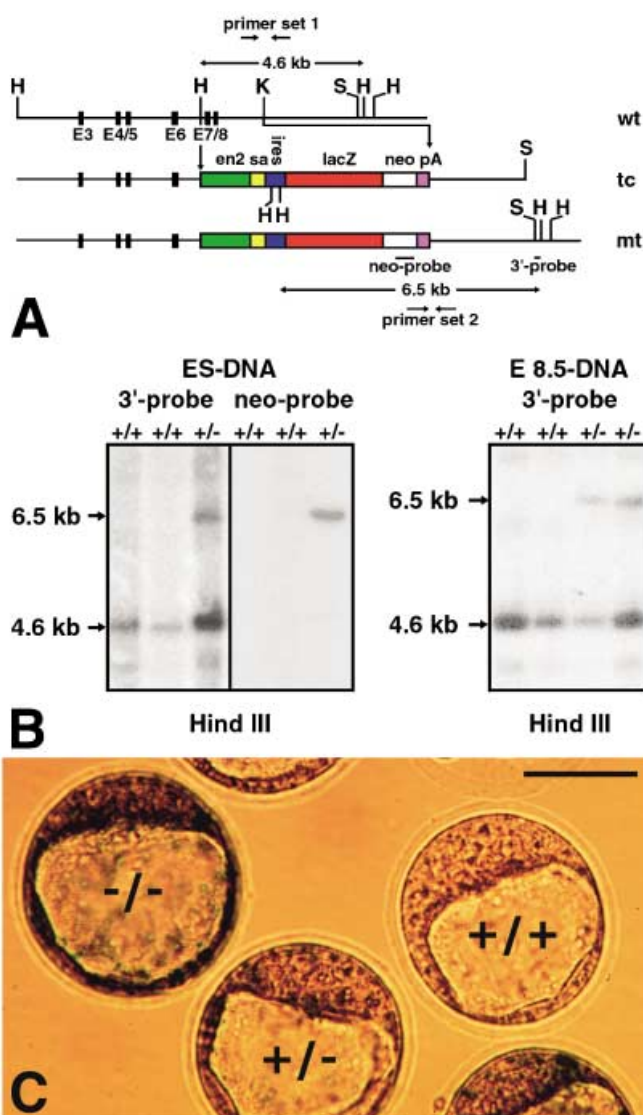
### Immunoblot analyses

Cells were washed twice with ice-cold PBS, incubated for 30 min on ice in buffer containing 5 mM EDTA, 5 mM EGTA, 1.5 M KCl, 10 mM Tris-HCl, pH 7.5, 1% (v/v) TritonX-100, 1 mM PMSF, and 1 mM DTT, and were homogenized with a tight-fitting Dounce homogenizer by 30 up and down strokes. Centrifugation for 30 min at 5000g yielded high-salt pellets that were washed twice with PBS and dissolved in loading buffer for standard SDS-PAGE.

## Results and Discussion

### Inactivation of DSG2 results in lethality of the periimplantation embryo

Five ES cell clones, each containing one inactivated DSG2 allele, were isolated by homologous recombination with a promoterless targeting vector (Fig. 1A, B). In these cells, exons 7 and 8 of the mutated DSG2 gene were substituted by a cassette conferring *lacZ* expression and neomycin resistance. The disruption of the DSG2 gene is expected to result in the production of a dysfunctional aminoterminal Dsg2 fragment terminating in the extracellular E2 domain at a position that corresponds to amino acid 229 of human Dsg2. ES cell clones 14 and 32 were used to generate male chimeras that were bred with either C57BL/6 or 129/Sv females. DSG2<sup>+/-</sup> animals were viable and fertile but failed to produce live DSG2<sup>-/-</sup> offspring for both ES cell derivatives in either genetic background.



**Fig. 1.** Strategy for homologous recombination of *DSG2* (A), analysis of ES cells and embryos by Southern blot hybridization (B), and morphology of early embryos from heterozygous intercrosses (C). (A) *DSG2* wild-type allele (wt), targeting construct (tc) and resulting mutated allele (mt). By homologous recombination, the 1.8-kb HindIII/KpnI wild-type fragment comprising exons 7 and 8 (E7/8) is exchanged for a promoterless cassette consisting of the intronic sequence from en2, a splice acceptor site (sa), an internal ribosomal entry site (ires) and a β-galactosidase gene (*lacZ*) fused to a neomycin-resistance gene (*neo*), followed by a polyadenylation site (pA). The position of probes, hybridizing fragments, and PCR primer sets are denoted. H, HindIII; K, KpnI; S, SphI. (B) Autoradiographs of Southern blot hybridization experiments identifying *DSG2*<sup>+/+</sup> and *DSG2*<sup>+/-</sup> ES cells and microdissected E8.5 embryos by using either an external 3'-probe or an internal transgene-specific probe (neo-probe) on HindIII-digested DNA. (C) Micrograph of blastocysts (E4.5) stained for β-galactosidase activity. The genotype of the embryos was determined by PCR after imaging. Note that there is no apparent difference in morphology between the genotypically different embryos. Bar: 50 μm.

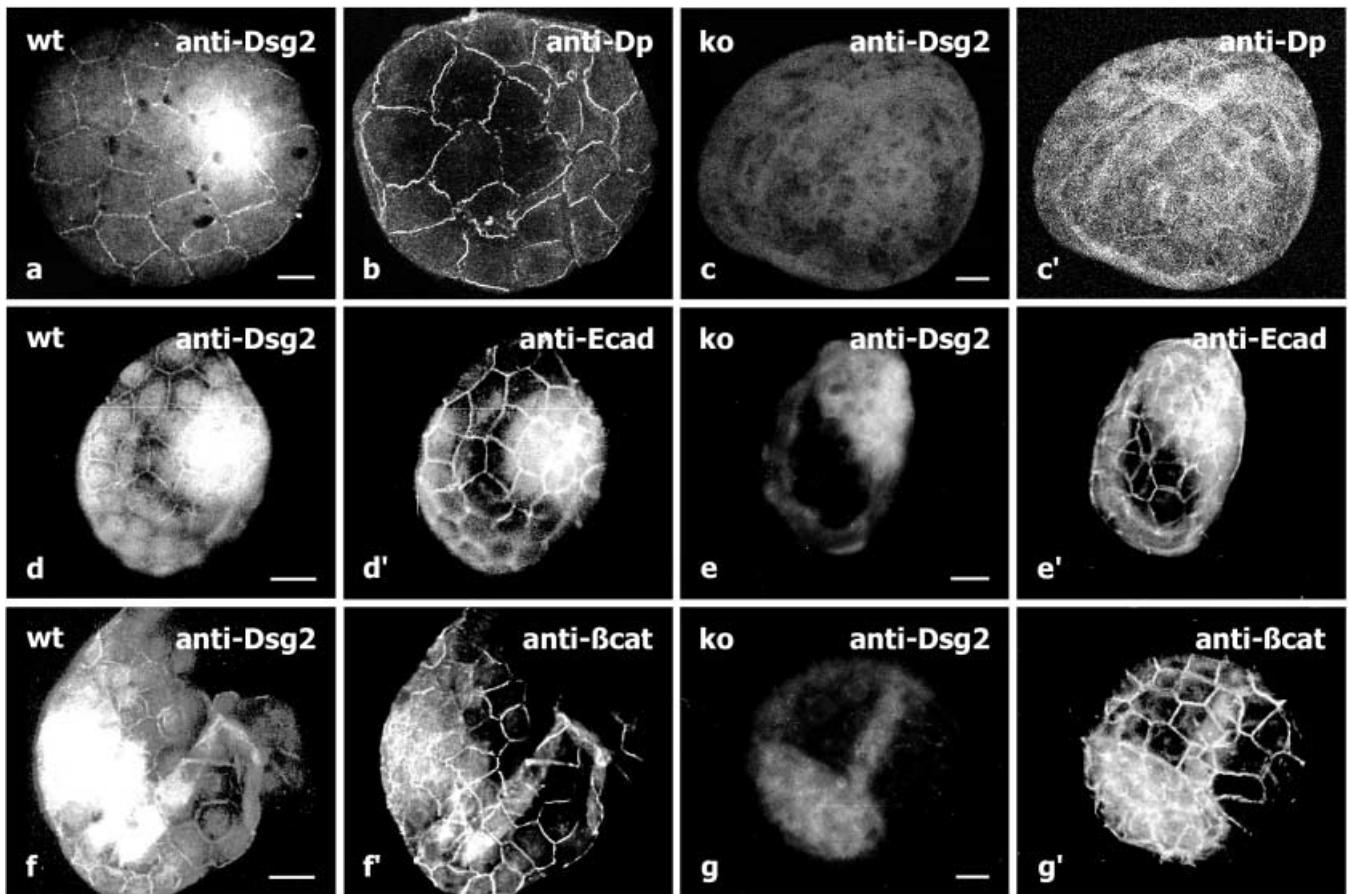
Furthermore, instead of the expected 2:1 ratio, an approximately even number of *DSG2*<sup>+/-</sup> and *DSG2*<sup>+/+</sup> mice (53 and 56 animals, respectively) was born from heterozygous intercrosses as determined by genomic Southern blot hybridization.

Further experiments were performed with ES cell clone 14 derivatives. First, embryos from *DSG2*<sup>+/-</sup> parents were examined to determine the time point of death. Between E8.5 and E18.5 a similar genotype distribution was noted as in new-born pups (13 *DSG2*<sup>+/+</sup>, 17 *DSG2*<sup>+/-</sup>; see also Fig. 1B). Since the small tissue amounts and the presence of contaminating maternal tissue made it impossible to perform reliable DNA analyses at earlier developmental time points, serial histological sections were prepared from E5.0 to E6.5 embryos for morphological evaluation. In many instances, decidual reaction was seen either with variable amounts of embryonic remnants exhibiting alterations in embryonic and trophoblast-derived regions or without any embryonic material (not shown). At E5.0, 37.5% (9/24) of decidual reactions were negative, at E5.5 34.5% (38/110), and at E6.5 41.7% (15/36), indicating that not only all *DSG2*<sup>-/-</sup> but also some *DSG2*<sup>+/-</sup> embryos did not survive implantation. In accordance, only 7 of 12 E5.5 embryos were *lacZ*-positive, i.e., they contained at least one targeted *DSG2* allele. Before implantation, however, two of eight E3.5 blastocysts were *DSG2*<sup>-/-</sup>, four *DSG2*<sup>+/-</sup>, and two *DSG2*<sup>+/+</sup> as determined by PCR. In addition, 61 of 352 E3.5 embryos that were cultivated for an additional day in vitro were *DSG2*<sup>-/-</sup> as determined by immunofluorescence microscopy with *Dsg2* antibodies. No morphological anomalies were observed at the blastocyst stage in any of the *lacZ*-positive *DSG2*<sup>+/-</sup> and *DSG2*<sup>-/-</sup> embryos (Fig. 1C). PCR analysis of either strongly or moderately *lacZ*-positive blastocysts before and after hatching confirmed that they were *DSG2*<sup>-/-</sup> and *DSG2*<sup>+/-</sup>, respectively.

Breeding of *DSG2*<sup>+/-</sup> and wild-type mice further showed that heterozygosity was sufficient to induce periimplantation lethality. The percentage of *DSG2*<sup>+/-</sup> embryos was 33.3% at E5.5 (n = 9), 32% at E6.5 (n = 25), and 22.6% at E8.5 (n = 31), whereas the expected Mendelian percentage of 50% was only found at E3.5 (n = 24). Negative decidual reactions were also noted at elevated rates after implantation (42/107), although some of them may have resulted from damage inflicted during preparation. Taken together, we conclude that all *DSG2*<sup>-/-</sup> embryos and a considerable proportion of *DSG2*<sup>+/-</sup> embryos die around implantation.

### Lethality of *DSG2*<sup>+/-</sup> mice is dependent on genetic background

To examine the influence of genetic background for the lethality of *DSG2*<sup>+/-</sup> mice, we crossed male *DSG2*<sup>+/-</sup> mice for several generations into an inbred 129/Sv or a C57BL/6 background. In both breeding schemes, the number of *DSG2*<sup>+/-</sup> offspring was considerably lower than expected. In the 129/Sv background, 28.23% of the offspring (n = 379) was *DSG2*<sup>+/-</sup> with no apparent changes between the F1 and F7 generations. In contrast, continued breeding with C57BL/6 mice resulted in increasing survival of *DSG2*<sup>+/-</sup> embryos reaching almost the expected ratio of 1:1 for *DSG2*<sup>+/+</sup> and *DSG2*<sup>+/-</sup> animals in the F10 generation. The percentage of *DSG2*<sup>+/-</sup> animals increased from 26.56% in F1 (n = 241) to 37.10% in F6 (n = 124), reaching 40.00% in F8 (n = 40) and 46.67% in F10 (n = 15). Heterozygous lethality was independent of whether the mutated allele was inherited from the male or female parent. These observations suggest that modifier



**Fig. 2.** Whole-mount confocal immunofluorescence microscopy of E3.5 blastocysts after an additional day in culture; detection of the desmosomal proteins Dsg2 (**a, c–g**; anti-Dsg2) and desmoplakin (**b, c'**; anti-Dp), and the non-desmosomal junction proteins E-cadherin (**d', e'**;

anti-Ecad) and  $\beta$ -catenin (**f, g'**; anti- $\beta$ cat) in wild-type (**a, b, d, d', f, f'**; wt) and  $DSG2^{-/-}$  (**c, c', e, e', g, g'**; ko) mice. Note the altered distribution of desmoplakin in the absence of Dsg2. Bars: 10  $\mu$ m.

genes suppress the lethality of  $DSG2$  heterozygotes in C57BL/6 mice.

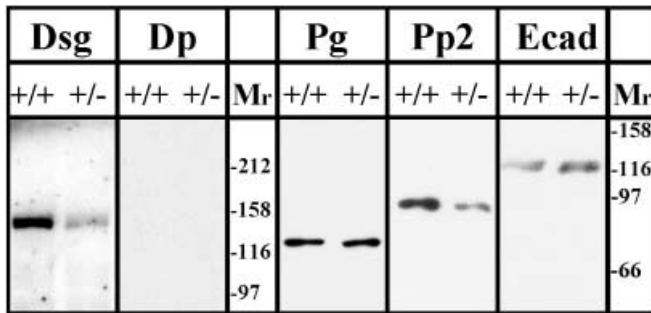
### Desmoplakin distribution is altered in $DSG2^{-/-}$ blastocysts

To identify possible differences in the morphologically normal-appearing  $DSG2^{-/-}$  blastocysts, immunofluorescence analyses of junctional proteins were carried out. The patchy staining of trophectoderm cell borders in wild-type blastocysts by using antibodies against Dsg2 or the desmosomal plaque protein desmoplakin is shown in Figure 2a, b. In the absence of Dsg2, however, very little desmoplakin was concentrated at cell borders and most was instead distributed throughout the cell (Fig. 2c'). In contrast, components of other adherens junctions, such as E-cadherin and  $\beta$ -catenin, remained localized to cell-cell borders in  $DSG2^{-/-}$  mice (compare Fig. 2d, f, f' with Fig. 2e, e', g, g').

### Dsg2 appears to be essential for embryonal stem cell growth

To improve our understanding of the function of Dsg2 for early development and the differentiation of ES cells, we tried to generate double-knockout ES cells in several ways. First, induction of gene conversion was attempted in the  $DSG2^{+/-}$  ES cells of clone 14 by increasing the concentration of G418 in the

medium to 4 mg/ml. However, none of the 100 resistant clones had lost the intact  $DSG2$  allele. Second, another targeting construct with a hygromycin selection cassette was introduced into ES cells of clone 14. Of the 100 hygromycin-resistant clones, none contained two mutated alleles. Third, ES cell lines were established from blastocysts obtained from  $DSG2^{+/-}$  intercrosses. ES cell line formation was successful in 15%. Of the 61 lines, 30 were  $DSG2^{+/-}$ , 31 were  $DSG2^{+/+}$ , but none was  $DSG2^{-/-}$ . When we examined the outgrowths of E3.5 embryos obtained from  $DSG2^{+/-}$  intercrosses, we found that after 3 days in culture, 36 of the 146 embryo derivatives were  $DSG2^{-/-}$ , but that by 6 days in culture, no viable  $DSG2^{-/-}$  cells were detectable, and that 32 of 57 outgrowths were  $DSG2^{+/+}$  and only 25  $DSG2^{+/-}$ . Taken together, these observations provide strong evidence that Dsg2 is necessary for ES cell viability and proliferation. This conclusion suggests, together with the observed formation of morphologically normal blastocysts, that the differentiation of ES cell equivalents is not disturbed but that the amplification of this cell population is compromised thereby resulting in inhibition of in vitro proliferation of inner cell mass-derived ES cells and in prevention of embryo maturation beyond implantation. Factors may be present in the in vivo situation that are provided by the developing trophectoderm and/or maternal tissues which compensate for the loss of Dsg2 until implantation.



**Fig. 3.** Immunoblot detection of junction proteins in wild-type ES cell clone E11 (+/+) and DSG2<sup>+/-</sup> ES cell clone E4 (+/-). Equal amounts of cytoskeletal fractions were separated by SDS-PAGE, blotted and reacted with antibodies detecting either Dsg, desmoplakin (Dp), plakoglobin (Pg), plakophilin 2 (Pp2), or E-cadherin (Ecad). Note the reduced expression of Dsg and plakophilin 2 in the mutated ES cells in contrast to the slight increase in E-cadherin. Position and relative molecular mass (Mr; units of 1000) of co-electrophoresed marker proteins are indicated.

### Dsg2 is involved in non-desmosomal junctional adhesion in embryonic stem cells

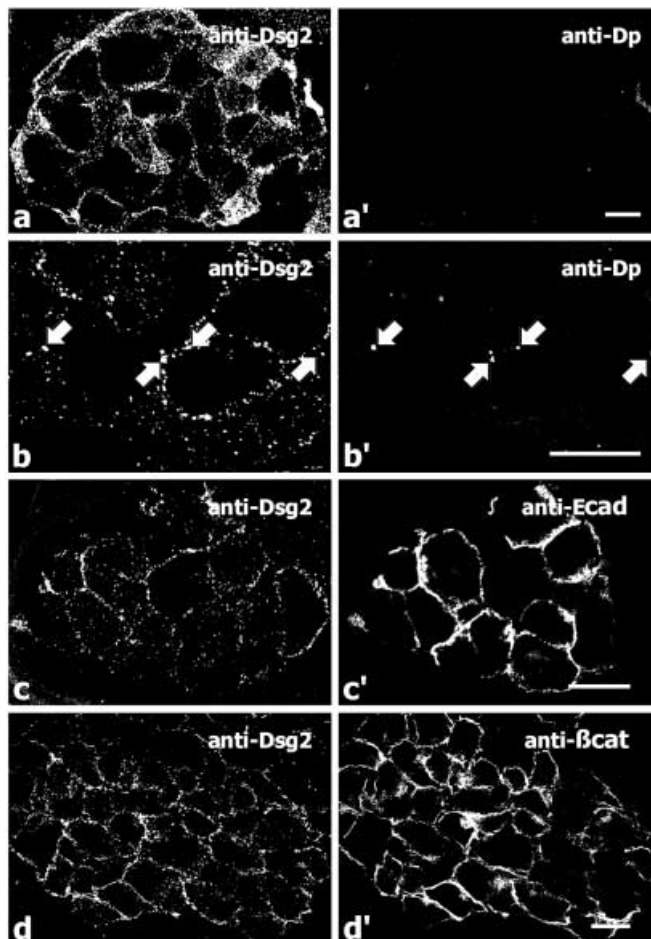
In an attempt to understand why Dsg2 is essential for ES cells, we examined its synthesis and distribution in these cells and compared it to that of desmosomal and other junctional proteins. By immunoblot analysis, we detected Dsg together with the ubiquitous plaque protein plakoglobin, and the dual-function desmosomal plaque and karyoplasmic protein plakophilin 2 (Fig. 3). In contrast, the obligatory desmosomal plaque protein desmoplakin could not be found in cytoskeletal fractions of wild-type ES cells (Fig. 3), indicating that ES cells cannot form mature desmosomes. Remarkably, in DSG2<sup>+/-</sup> cells, we noticed not only a reduction of Dsg – as was expected – but also a decrease of plakophilin 2 (Fig. 3), showing that an interrelationship exists between Dsg2 and plakophilin 2. In addition, a slight increase in the level of the non-desmosomal adhesion molecule E-cadherin was seen in DSG2<sup>+/-</sup> ES cells, giving further evidence that the overall balance of junctional polypeptides is disturbed (Fig. 3).

Since significant amounts of desmoplakin were lacking in ES cells, we wanted to know if Dsg2 is concentrated in alternative, non-desmosomal adhesion sites and/or if it was located in alternative cellular compartments. By immunofluorescence microscopy, Dsg2 was detected at cell borders in small puncta showing that it is present in clusters and not diffusely throughout the cell surface (Fig. 4a–d). As expected, most cells were completely negative for desmoplakin (Fig. 4a'). However, occasionally sparse but distinct desmoplakin staining was noted in some cells where it coincided with Dsg2-positive spots (Fig. 4b, b'). Most ES cells were positive for the non-desmosomal junction molecules E-cadherin and  $\beta$ -catenin (Fig. 4c', d'). The fluorescence co-localized in part with Dsg2, although the overall distribution differed significantly (Fig. 4c, c' and Fig. 4d, d').

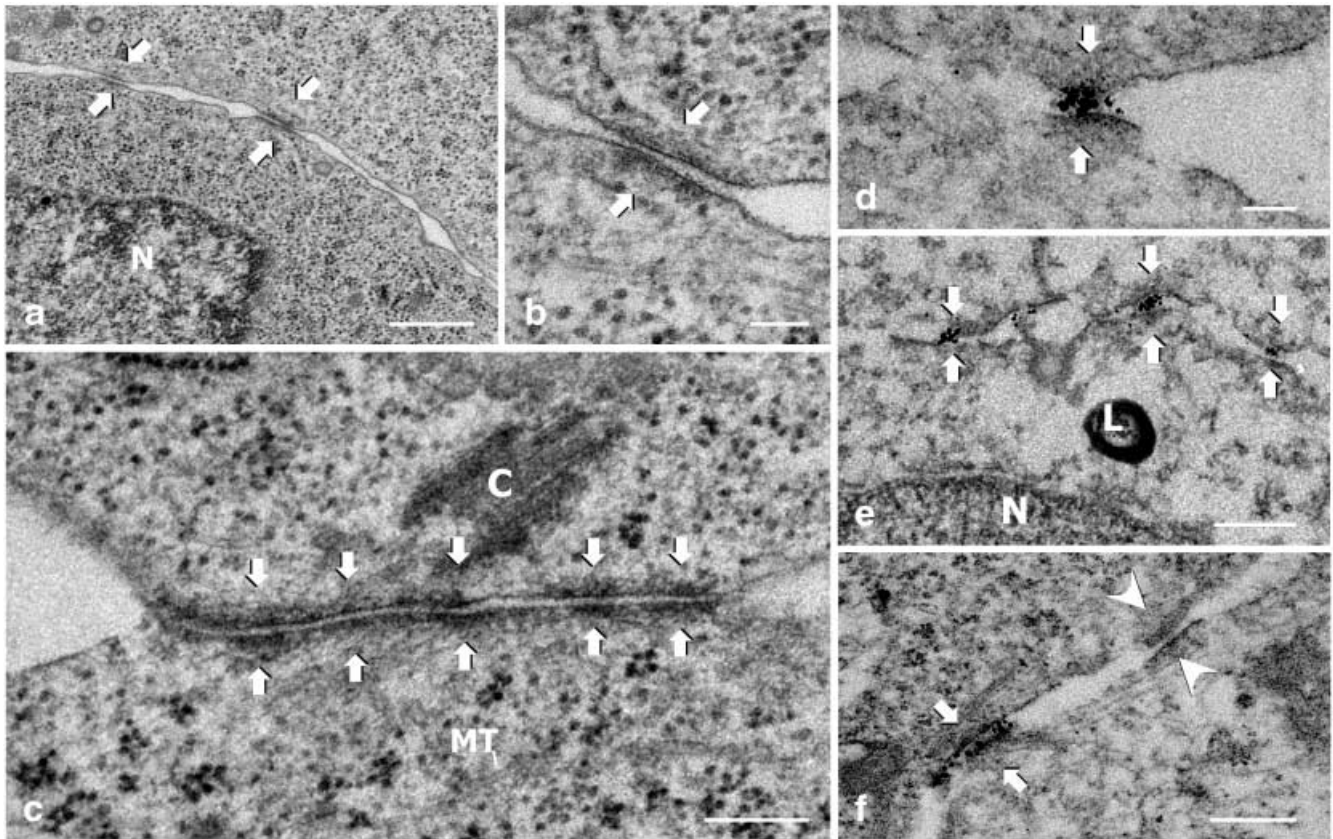
To identify and characterize non-desmosomal junctions in ES cells that contain Dsg2, we performed electron microscopy. We found many small cell-cell contact sites that were associated with small plaques lacking prominent filament bundles, although filamentous material was often seen in the vicinity (Fig. 5a–c). By immunoelectron microscopy we found that some of these junctions were labeled by Dsg2 antibodies although others with similar morphology were negative (Fig. 5d–f). Experiments are underway to determine the precise molecular composition of these newly identified and hitherto unknown junctions. It is tempting to speculate that they correspond to early stages of desmosomes resembling those described as nascent desmosomes in the trophectoderm of early embryos (Jackson et al., 1980, 1981; Fleming et al., 1991, 1994). Taken together, these results suggest that Dsg2 participates in non-desmosomal junctional adhesion of ES cells.

In conclusion, our observations show that Dsg2 fulfils a crucial role during early embryonic development and that this function is independent of mature desmosomes. This function, however, appears to be different from that of the desmosomal plaque protein desmoplakin which is also essential for early postimplantation development (Gallicano et al., 1998).

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**Fig. 4.** Double immunofluorescence microscopy of ES cells detecting Dsg2 (a–d; anti-Dsg2) together with either desmoplakin (a', b'; anti-Dp), E-cadherin (c'; anti-Ecad), or  $\beta$ -catenin (d'; anti- $\beta$ cat). Note the peripheral punctate staining pattern of Dsg2 in the absence of desmoplakin (a, a'), although single spots in peripheral regions of ES colonies are positive for both (b, b'). Bars: 10  $\mu$ m.



**Fig. 5.** Characterization of junctions in undifferentiated wild-type ES cells by electron microscopy. (a–c) Typical cell-cell contacts in undifferentiated wild-type ES cells (arrows). C, centriole; MT, microtubule; N, nucleus. (d–f) Immunoelectron microscopy of wild-type ES

cells detecting Dsg2. Note the specific labeling of most (arrows), but not all (arrowheads in f) cell-cell contacts. L, lysosome; N, nucleus. Bars: 500 nm in a; 200 nm in b–d; 250 nm in e, f.

## References

- Allen, E., Yu, Q.-C., Fuchs, E. (1996): Mice expressing a mutant desmosomal cadherin exhibit abnormalities in desmosomes, proliferation, and epidermal differentiation. *J. Cell Biol.* **133**, 1367–1382.
- Amagai, M., Matsuyoshi, N., Wang, Z. H., Andl, C., Stanley, J. R. (2000): Toxin in bullous impetigo and staphylococcal scalded-skin syndrome targets desmoglein 1. *Nat. Med.* **6**, 1275–1277.
- Chidgey, M., Brakebusch, C., Gustafsson, E., Cruchley, A., Hail, C., Kirk, S., Merritt, A., North, A., Tselepis, C., Hewitt, J., Byrne, C., Fassler, R., Garrod, D. (2001): Mice lacking desmocollin 1 show epidermal fragility accompanied by barrier defects and abnormal differentiation. *J. Cell Biol.* **155**, 821–832.
- Collins, J. E., Fleming, T. P. (1995): Epithelial differentiation in the mouse preimplantation embryo: making adhesive cell contacts for the first time. *Trends Biochem. Sci.* **20**, 307–312.
- Collins, J. E., Lorimer, J. E., Garrod, D. R., Pidsley, S. C., Buxton, R. S., Fleming, T. P. (1995): Regulation of desmocollin transcription in mouse preimplantation embryo. *Development* **121**, 743–753.
- Eshkind, L. G., Leube, R. E. (1995): Mice lacking synaptophysin reproduce and form typical synaptic vesicles. *Cell Tissue Res.* **282**, 423–433.
- Fleming, T. P., Garrod, D. R., Elsmore, A. J. (1991): Desmosome biogenesis in the mouse preimplantation embryo. *Development* **112**, 527–539.
- Fleming, T. P., Butler, L., Lei, X., Collins, J., Javed, Q., Sheth, B., Stoddart, N., Wild, A., Hay, M. (1994): Molecular maturation of cell adhesion systems during mouse early development. *Histochemistry* **101**, 1–7.
- Gallicano, G. I., Kouklis, P., Bauer, C., Yin, M., Vasioukhin, V., Degenstein, L., Fuchs, E. (1998): Desmoplakin is required early in development for assembly of desmosomes and cytoskeletal linkage. *J. Cell Biol.* **143**, 2009–2022.
- Hanakawa, Y., Amagai, M., Shirakata, Y., Sayama, K., Hashimoto, K. (2000): Different effects of dominant negative mutants of desmocollin and desmoglein on the cell-cell adhesion of keratinocytes. *J. Cell Sci.* **113**, 1803–1811.
- Hogan, B., Beddington, R., Costantini, F., Lacy, E. (1994): Manipulating the mouse embryo. A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 373–375.
- Hunt, D. M., Rickman, L., Whittock, N. V., Eady, R. A. J., Simrak, D., Dopping-Hepenstal, P. J. C., Stevens, H. P., Armstrong, D. K. B., Hennies, H. C., Küster, W., Hughes, A. E., Arnemann, J., Leigh, I. M., McGrath, J. A., Kelsell, D. P., Buxton, R. S. (2001): Spectrum of dominant mutations in the desmosomal cadherin desmoglein 1, causing the skin disease striate palmoplantar keratoderma. *Eur. J. Hum. Genet.* **9**, 197–203.
- Jackson, B. W., Grund, C., Schmid, E., Burki, K., Franke, W. W., Illmensee, K. (1980): Formation of cytoskeletal elements during mouse embryogenesis. Intermediate filaments of the cytokeratin type and desmosomes in preimplantation embryos. *Differentiation* **17**, 161–179.
- Jackson, B. W., Grund, C., Winter, S., Franke, W. W., Illmensee, K. (1981): Formation of cytoskeletal elements during mouse embryogenesis. II. Epithelial differentiation and intermediate-sized filaments in early postimplantation embryos. *Differentiation* **20**, 203–216.
- King, I. A., Angst, B. D., Hunt, D. M., Kruger, M., Arnemann, J., Buxton, R. S. (1997): Hierarchical expression of desmosomal

- cadherins during stratified epithelial morphogenesis in the mouse. *Differentiation* **62**, 83–96.
- Koch, P. J., Goldschmidt, M. D., Zimbelmann, R., Troyanovsky, R., Franke, W. W. (1992): Complexity and expression patterns of the desmosomal cadherins. *Proc. Natl. Acad. Sci. USA* **89**, 353–357.
- Koch, P. J., Mahoney, M. G., Ischikawa, H., Pulkkinen, L., Uitto, J., Shultz, L., Murphy, G. F., Whitaker-Menezes, D., Stanley, J. R. (1997): Targeted disruption of the pemphigus vulgaris antigen (desmoglein 3) gene in mice causes loss of keratinocyte cell adhesion with a phenotype similar to pemphigus vulgaris. *J. Cell Biol.* **137**, 1091–1102.
- Koch, P. J., Mahoney, M. G., Cotsarelis, G., Rothenberger, K., Lavker, R. M., Stanley, J. R. (1998): Desmoglein3 anchors telogen hair in the follicle. *J. Cell Sci.* **111**, 2529–2537.
- Kowalczyk, A. P., Bornslaeger, E. A., Norvell, S. M., Palka, H. L., Green, K. J. (1999): Desmosomes: Intercellular adhesive junctions specialized for attachment of intermediate filaments. *Int. Rev. Cytol.* **185**, 237–302.
- Lorimer, J. E., Hall, L. S., Clarke, J. P., Collins, J. E., Fleming, T. P., Garrod, D. R. (1994): Cloning, sequence analysis and expression pattern of mouse desmocollin 2 (DSC2), a cadherin-like adhesion molecule. *Mol. Membr. Biol.* **11**, 229–236.
- Marcozzi, C., Burdett, I. D. J., Buxton, R. S., Magee, A. I. (1998): Coexpression of both types of desmosomal cadherin and plakoglobin confers strong intercellular adhesion. *J. Cell Sci.* **111**, 495–509.
- Norvell, S. M., Green, K. J. (1998): Contributions of extracellular and intracellular domains of full length and chimeric cadherin molecules to junction assembly in epithelial cells. *J. Cell Sci.* **111**, 1305–1318.
- Roberts, G. A., Burdett, I. D., Pidsley, S. C., King, I. A., Magee, A. I., Buxton, R. S. (1998): Antisense expression of a desmocollin gene in MDCK cells alters desmosome plaque assembly but does not affect desmoglein expression. *Eur. J. Cell Biol.* **76**, 192–203.
- Runswick, S. K., O'Hare, M. J. O., Jones, L., Streuli, C. H., Garrod, D. R. (2001): Desmosomal adhesion regulates epithelial morphogenesis and cell positioning. *Nat. Cell Biol.* **3**, 823–830.
- Schäfer, S., Koch, P. J., Franke, W. W. (1994): Identification of the ubiquitous human desmoglein, Dsg2, and the expression catalogue of the desmoglein subfamily of desmosomal cadherins. *Exp. Cell Res.* **211**, 391–399.
- Schäfer, S., Stumpp, S., Franke, W. W. (1996): Immunological identification and characterization of the desmosomal cadherin Dsg2 in coupled and uncoupled epithelial cells and in human tissues. *Differentiation* **60**, 99–108.
- Stanley, J. R. (1993): Cell adhesion molecules as targets of autoantibodies in pemphigus and pemphigoid, bullous diseases due to defective epidermal cell adhesion. *Adv. Immunol.* **53**, 291–325.
- Strnad, P., Windoffer, R., Leube, R. E. (2001): In vivo detection of cytokeratin filament network breakdown in cells treated with the phosphatase inhibitor okadaic acid. *Cell Tissue Res.* **306**, 277–293.
- Theis, D. G., Koch, P. J., Franke, W. W. (1993): Differential synthesis of type 1 and type 2 desmocollin mRNAs in human stratified epithelia. *Int. J. Dev. Biol.* **37**, 101–110.
- Tian, Q. (2000): Molekularbiologische Charakterisierung des menschlichen Desmoglein 2 (DSG2)-Gens und Ausschaltung dieses Genes bei der Maus durch homologe Rekombination. PhD thesis, University Heidelberg, pp. 1–157.
- Troyanovsky, S. M., Leube, R. E. (1998): Molecular dissection of desmosomal assembly and intermediate filament anchorage. *Subcell. Biochem.* **31**, 263–289.
- Troyanovsky, S. M., Eshkind, L. G., Troyanovsky, R. B., Leube, R. E., Franke, W. W. (1993): Contributions of cytoplasmic domains of desmosomal cadherins to desmosome assembly and intermediate filament anchorage. *Cell* **72**, 561–574.
- Troyanovsky, S. M., Troyanovsky, R. B., Eshkind, L. G., Krutovskikh, V. A., Leube, R. E., Franke, W. W. (1994a): Identification of the plakoglobin-binding domain in desmoglein and its role in plaque assembly and intermediate filament anchorage. *J. Cell Biol.* **127**, 151–160.
- Troyanovsky, S. M., Troyanovsky, R. B., Eshkind, L. G., Leube, R. E., Franke, W. W. (1994b): Identification of amino acid sequence motifs in the desmosomal glycoprotein, desmocollin, that are required for plakoglobin binding and plaque formation. *Proc. Natl. Acad. Sci. USA* **91**, 10790–10794.
- Windoffer, R., Borchert-Stuhlträger, M., Leube, R. E. (2002): Desmosomes: interconnected calcium-dependent structures of remarkable stability with significant integral membrane protein turnover. *J. Cell Sci.* **115**, 1717–1732.