Eukaryotic cells contain internal membrane compartments that are functionally connected by a
dynamic series of trafficking pathways. Transport between compartments is carried out by distinct
classes of coated vesicles of which the clathrin-coated vesicle (CCV) is one of the best understood
at the structural level. However, there is a need for the detailed in-vivo function of clathrin in
higher vertebrates, because the vast majority of our knowledge is mainly derived from in-vitro
studies combined with a limited number of in-vivo experiments. These are either are not
exclusively specific for clathrin-mediated transport pathways or are limited in their applicability
for quantitative analysis. Genetic approaches have been attempted, but were restricted to single
celled eukaryotes, which are only distantly related to higher vertebrates.

We have exploited the high rate of homologous recombination exhibited by the chicken pre-B cell
line DT40 to delete the alleles for clathrin heavy-chain and and replace them with human clathrin
under the control of the tetracycline-regulatable expression system (Tet-off). This allowed clathrin
expression to be manipulated by the addition of doxycycline to the media, which enabled us to
carry out the first functional study of vertebrate clathrin using gene targeting. In the initially
produced cell line, designated DKO-S, full repression of clathrin synthesis triggered apoptosis, as
demonstrated by the three hallmarks, namely DNA-fragmentation into multiples of 180-200bp,
formation of apoptotic bodies and caspase activation. Clathrin depletion did not affect the pro-
apoptotic stress-activated signalling pathways involving the key regulators p38 or JNK, but did
reduce the activity of Akt-mediated signalling and slightly increased proliferative signalling via
MAP kinase. We propose that clathrin removal perturbed the integration of these two signal
transduction pathways in DT40.

We also report the isolation and partial characterisation of a variant, designated DKO-R, which
was derived from the DKO-S cell line. It retained the ability to regulate clathrin expression with
the Tet-system, but failed to undergo apoptosis. Significantly, the signal-transduction pathway
involving Akt was no longer sensitive to clathrin withdrawal. In fact both, the Akt- and the MAP
kinase-mediated pathways, were hyper-stimulated in DKO-R cells, which rendered these cells less
dependent on external serum factors for survival than DKO-S cells. Although the basic apoptotic
machinery was still functional, the constitutive activation of the survival and proliferative
signalling pathways irrespective of clathrin expression uncoupled the link between membrane
traffic and apoptosis in DKO-R cells. We confirmed that the difference between the two cell lines
was solely due to alterations in the signal transduction pathways, rather than a change of
membrane traffic as an adaptation.
Since DKO-R cells survive in the absence of clathrin, we used this cell line to examine effects of clathrin depletion on vesicle transport. The rate of receptor-mediated endocytosis of transferrin was greatly inhibited by approximately 80 percent as well as fluid-phase uptake of horseradish peroxidase. Caveolin-1, which was a candidate for non-clathrin-mediated vesicle transport, was expressed neither in DKO-S nor in DKO-R cells independent of CHc-levels. Taken together these results revealed that clathrin-mediated endocytosis is the major endocytic route in DT40. The use of our model system also enabled me to elucidate for the first time in-vivo that clathrin is partially involved in the recycling of membrane receptors from the endosomal compartment. Clathrin depletion slightly reduced the fraction of internalised transferrin that was subsequently recycled as well as the rate of recycling by ≈30 percent. In the absence of clathrin, the binding of the heterotetrameric adaptors AP-1 and AP-2 and the only recently discovered monomeric adaptor GGA1 to their respective target membranes (TGN for AP-1 and GGA-1, plasma membrane for AP-2) was significantly enhanced, but the distribution of the AP-3 adaptor complex was unaffected. Clathrin suppression also changed the distribution of the cation-independent mannose-6-phosphate receptor, which has been postulated to direct lysosomal transport, to a more intense vacuolar staining pattern. Surprisingly however, clathrin removal had no effect on the morphology of dense-core lysosomes or the intracellular distribution of soluble and membrane-bound lysosomal proteins, implying the existence of a clathrin-independent pathway of lysosome biogenesis.

In conclusion, we have exploited the unique properties of the DT40 cell line to apply the power of gene targeting in the study of vertebrate clathrin. By replacing the endogenous clathrin-heavy chain gene with the human homologue under the control of a regulatable promotor, we have produced a "clean" and highly flexible experimental in-vivo model for the first time since clathrin's discovery in 1975. Our results raise important and unexpected questions about the molecular mechanism and general function of this protein in vesicle transport and lysosome biogenesis. Additionally, our data suggests that in DT40 clathrin plays a crucial role in maintaining the integrity of anti-apoptotic survival pathways and hence we have identified a novel connection between membrane traffic and programmed cell death. This link provides evidence for a potential role of deregulated clathrin function and possibly membrane transport in oncogenic transformations. Our model system will be very useful to address these and other aspects in great detail.