ExcellGene

# FROM CORN TO CHO TRANSPOSON-TECHNOLOGY

## Maturing a concept over decades

### Part 2 of 4



# CHO cells have endogenous retroviral DNA elements producing retrovirus-like particles

## Summary of the previous chapter:

In the 1970s, a Genetics student in Germany read 1950/1953 publications of Dr Barbara McClintock. These groundbreaking papers, challenged the current thinking in classical genetics at the time, made her win the Nobel prize 30 years after the publications, at the age of 81 and that as a single recipient! When studying the genetics of common corn, she identified unique genetic elements, now named jumping genes, mobile genetic elements, or transposons.

These DNA elements were translocating from one position on chromosomes to another one, while cells in an embryonic corn seed were dividing to grow. Eventually scientists learned that such DNA sequences arose early in evolution as “DNA parasites”. The elements apply a diversity of principles to move in and out of genomic DNA and evolved within genomes to be efficient over millennia. These and their non-functional remnants constitute more than 90% of all DNA in humans, animals, and plants.

# October 1986 in California– questions on CHO cells and their products

The department directors and group leaders of Cell Line Development and Process Sciences met to discuss an important topic – retrovirus-like DNA elements in animal cells and potential infection risks from such cells. The AIDS epidemic, exploding in San Francisco at the time, made the public aware of retroviruses. Their potential for serious disease and death was and is frightening. And as scientists explored, they found genetic DNA elements resembling the RNA of such viruses in many species. Diagrams in Fig. 1.1 and 1.2 show steps of the life cycle of retroviruses. Critically, a copy of the RNA genome, a pro-viral DNA becomes inserted into the genome (one of the chromosomes) of the host cells using a virus encoded "integrase" (1). Once integrated, this DNA transcribes into messenger RNA (mRNA) and leads to the formation of new viral particles. The new particles will infect other cells (also assuring the integration and persistence of the pro-viral DNA). For an HIV-infected person, this is terrifying because the DNA of the AIDS virus remains in the body for life. Retroviral DNA elements will also be inherited from parent to offspring if copies entered cells that generate sperm or maternal oocytes. These genetic elements, called "Endogenous Retroviral Sequences (ERS)" are not rare – thousands of them have been found in genomes of many species, belonging to the class of mobile genetic elements discussed in Part 1 of this text.

# Endogenous Retroviral Sequences (ERS) in CHO cells?

Pertinent questions were raised: Do CHO cells have active endogenous retroviral sequences? Would it be possible that these cells produce retroviruses particles that could end up in a product made with them?

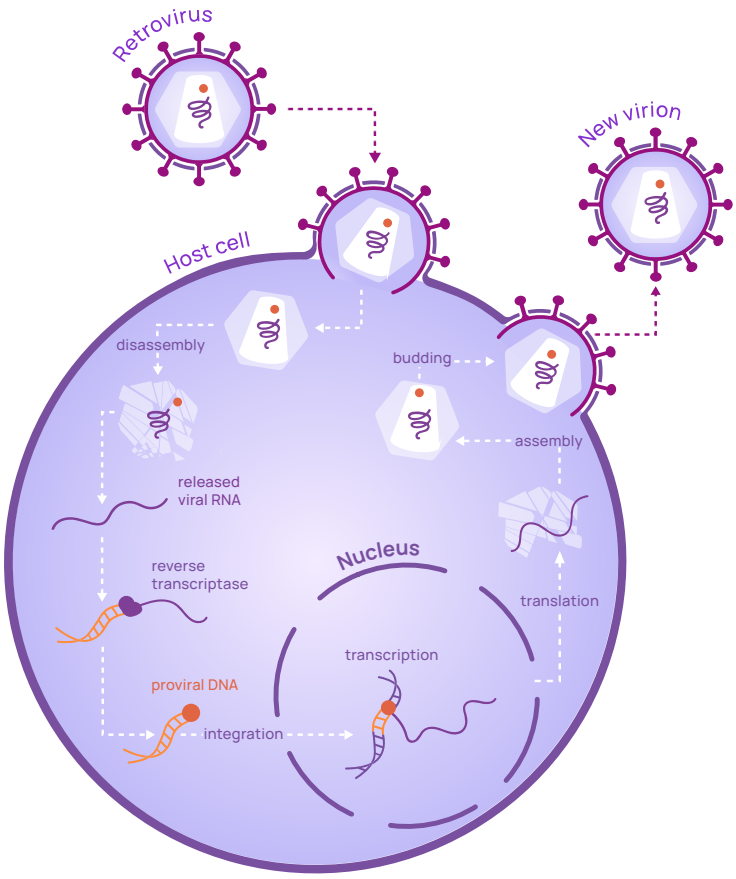


**Fig. 1.1**

Diagram of the life cycle of retroviruses: The RNA genome of the virus is "reverse transcribed" into DNA and the resulting pro-viral DNA is then integrated into one of the chromosomes of the infected cell. Transcription (RNA) and translation (protein) from this DNA results eventually into new virus particles.

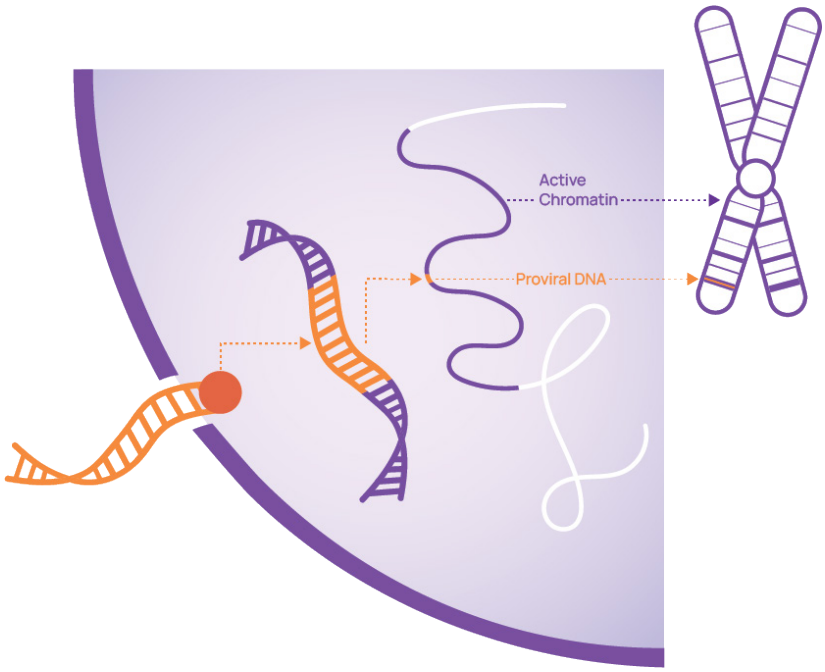


**Fig. 1.2**
The steps of integration of the proviral DNA into the genomic DNA of the host cell by a virus-encoded "integrase" is shown within the nuclear environment, where DNA has just entered through a nuclear pore. The integrase has a preference for active chromatin. Blue indicates active chromatin, white indicates heterochromatin, considered not encoding genes for proteins. The compacted chromosome diagram on the upper right shows that active chromatin is only a small part of all DNA.

The mid-eighties saw pioneering CHO cultures growing in large-scale stainless-steel bioreactors to produce a complex protein. The product, a therapeutic agent that could remove blood clots in arteries, was to be injected into patients suffering from a heart attack (2). The questions raised in this context were troubling and of a major concern, particularly for a relatively small biotech company. In this context, a team of three from the analytical and cell line development departments was addressed that included the recently hired former student from Germany: We must assume that CHO cells contain such sequences. How many? What do they do there? Do they encode functional virus particles? Are they infective? Please find out more.

## One thousand DNA fragments of ERS in the CHO genome

Mobile genetic elements, including endogenous retroviral sequences can accumulate in copy number in the genome of higher animals (chapter 1). A publication on ERS' in Syrian Hamsters had just come out (3): The Japanese research group was kind and shared cloned DNA of these sequences with the team of three to help with the CHO work. A few weeks later, the answers came in: Yes – with widely used techniques to pick up DNA sequences in the genome of a related species – similar ERS fragments were found in CHO DNA and in extracted mRNA transcripts from CHO cells. Thus, at least some genome elements were active. Three different sequence "families" were identified in CHO cells (4,5) in comparison to the Syrian hamster reference DNA. Similarities in sequence ranged from 40-70%. As with other ERS' in mouse, human and the Syrian hamster, striking information was found in the CHO genome: For functional, ie. gene encoding DNA, three nucleotides at a time are read in any DNA over long stretches (more than 100 base pairs) to generate a "reading frame". In CHO-ERS' frequent STOP codons were found in all three reading frames within the putative genomic DNA of a retrovirus so that none of the cloned ERS-DNA could generate functional RNA molecules for the virus. At least for those cloned ERS' no active virus could ever be made from them. However, some of the transcribed RNAs were made as larger molecules with several hundreds of nucleotides. Yet even these molecules were non-functional: Parts of the regions in the genome of retroviruses, indicated as "gag" and "env" appeared missing (Fig. 2), i.e.

short stretches of DNA had “open reading frames”, interrupted by gaps. This meant that the transcription of the messenger RNA was initiated, and an mRNA molecule was produced, but this mRNA could not result in a functional virus. Thus, none of the cloned CHO-ERS’, including the cloned and analyzed mRNA molecules provided evidence for the generation of functional retroviruses. The overall conclusion of the work was then that many of the ERS copies in the CHO genome had accumulated mutations over millennia in hamsters and their predecessor species deleting functionality.
From a risk perspective, this was assuring.

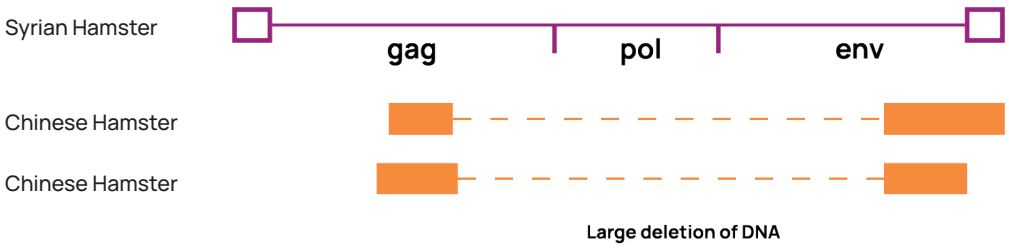


**Fig. 2**
Chinese Hamster Endogenous Retroviral (A-type) sequences showing short stretches of “readable” DNA (orange boxes) and large deletions (striped) in a segment of a putative retroviral genome, compared to a full-length Syrian Hamster retroviral sequence. The figure is modified from Ref 4.

## Could hidden CHO-ERS’ pose a risk?

But obviously this was not enough – as a reminder, only a few DNA-fragments had been sequenced. At the time it was not possible to sequence an entire CHO genomey because the methods for doing so were invented only many years later, with the first human genome sequence published in 2001 (6). However, a well-established method allowed to verify the presence of at least 1000 ERS-like elements, representing three different ERS families in the CHO genome. Thus, one could not exclude some ERS’ lurking in the background fully capable of producing active and infective retroviruses. Decades later, one CHO-ERS family was analyzed by genome sequencing and proteomic annotation, and this family was found to be present at about 200 copies (7,8). Among these 200 copies was at least one that appeared to be capable of constructing a complete retrovirus.

### Virus-like particles

During the mid 1980s at the company, further work was initiated to look directly into CHO cells, in the hope to see structures that resembled retroviruses. To do this, embedded CHO cells were cut into thin slices, and analyzed by Transmission Electron Microscopy (TEM). Eventually, after looking at numerous slices, a few were found that exhibited a small number of structures of the right size and similar to retrovirus particles seen before in mice and cell lines derived from them (A-type and C-type particles). In CHO, C-type particles are apparently assembling into more complete retrovirus structures and this was verified decades later by the publications

mentioned above (7,8). The smaller A-type particles were not found at the membrane of cells but remained within the cytoplasmic environment. For comparison, an TEM image of A-type particles from a mouse cell line is shown in Fig. 3.

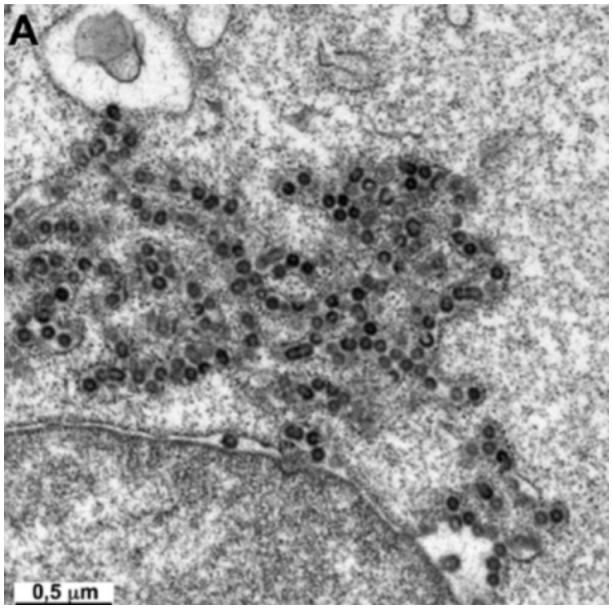


**Fig. 3**
Intracisternal A-type particles in a mouse cell line (from P. Rongeard (2008) Biol. Cell 100, 491-501).

## Virus inactivating steps

After numerous additional experiments, including tests showing convincingly that CHO-derived preparations lacked any virus infectivity in a diversity of sensitive cell culture systems, data were shared with the US-FDA. The agency agreed that CHO-derived virus particles were unlikely to pose a risk to patients with a life-threatening disease requiring the treatment with the therapeutic protein in clinical trials. However, to be on the safe side, all fluids of therapeutic protein were exposed to conditions during the purification process, so-called "virus-inactivation steps", that would inactivate viruses before the product containing liquids were filled into containers for therapy (Fig. 4).

## Reverse transcriptase

As reverse transcriptase (RT) (Fig. 1) is a critical component of the life cycle of retroviruses, tests were also developed to look for this enzyme in CHO cells – and yes, RT activity at very low levels was eventually verified. It is therefore reasonable to assume that functional reverse transcriptase activity can use any full-length, but also shortened (by deletions) and even entirely non-functional messenger RNA derived from ERS elements to re-integrate additional copies into the genome – this being a mechanism of the spread of "parasitic DNA" in CHO cells.

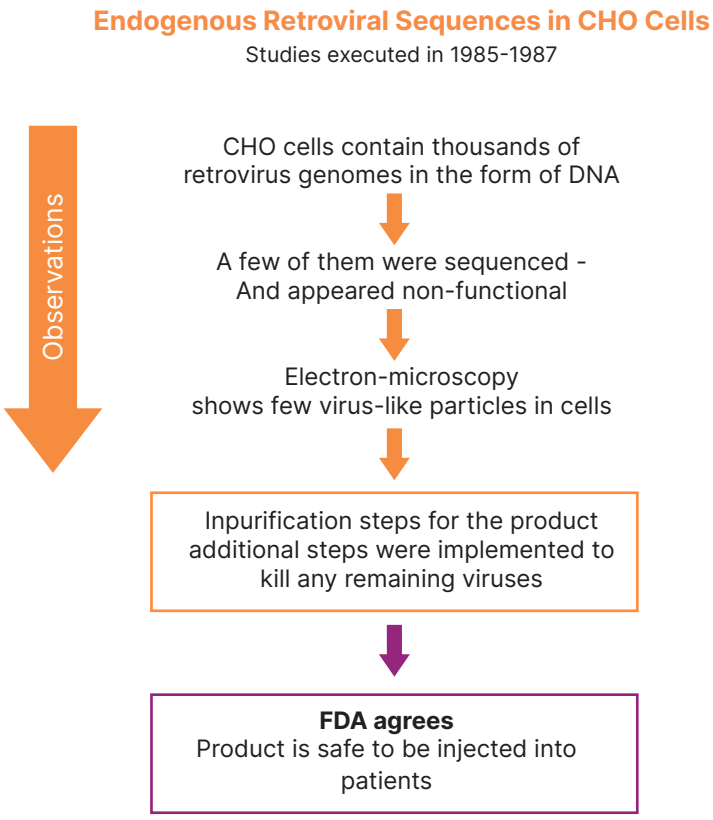


**Fig. 4**
Steps towards the safe use of CHO cells containing endogenous retroviral sequences.

## Abundant presence of ERS in animals

Today we know: Human DNA contains up to 8% of DNA derived from ancient retroviruses. They can be found in all vertebrates, including fish. This indicates a history of potential infections with such “DNA parasites” for more than 500 million years (9). Table 1 shows data extracted and slightly modified from the publication summarizing copy numbers and percentage of presence in the genomes of ERS in a diversity of animals, including fish and humans.

| | Size of Genome (Mbp) | % of total genome | Copy number |
|---|---|---|---|
| Frog | 1358 | 0.1 | 2259 |
| Human | 3321 | 8 | 496134 |
| Chicken | 1051 | 1.4 | 30420 |
| Coelacanth | 2861 | 0.006 | 673 |
| Zebrafish | 1413 | 0.76 | 30723 |
| Trout | 1878 | 0.09 | 3135 |
| Platyfish | 730 | 0.13 | 3065 |

Table 1:
Endogenous retroviral sequences, copy numbers and percentage in the respective genomic DNA of indicated animals (Mbp = million basepairs).

The differences in copy numbers and the space occupancy these elements take in the genome of animals from fish to humans are remarkable and are so far poorly explained. In humans nearly half a million of ERS fragments were found, even if many of them are very short fragments of a potential retrovirus DNA (10).
The table establishes that ERS have entered the germline of species frequently and are passed from parents to offspring over millennia. As with any DNA, many of these sequences are subject to point mutations and other genetic modifications such as deletion, duplications, etc., since they do not convey in general a selective advantage. What we observe today, are relics of a long history of infections in animals and humans, a field of study called paleovirology (11).

## A safe path for CHO cells as production hosts

CHO cells are preferred producer cell lines for therapeutic proteins in comparison to other mammalian host systems. For example, mouse derived cells, initially considered, were never used to produce pharmaceutically relevant proteins since some of these cells produce 100 to 1000-fold more viral particles (12). At least one family of ERS in the mouse genome contain sequences capable of producing infectious retroviruses (13). The overall insights obtained at the biotech company in California on CHO-ERS was therefore helpful in a general sense. CHO cells had taken a major barrier and seemed safe for manufacturing of therapeutic proteins. However, the assumption of a superior safety profile does not eliminate the need for testing and steps to eliminate any risks. The assumption that we know enough of the complexity of CHO genomes is presumptuous. The perpetually changing genetic composition of CHO cells as immortalized cells (14, 15) or even inherent “jumping gene” activity and recombination may occasionally result in a more profound production of such particles of which a small fraction could co-purify with the product of interest with unpredictable consequences.
Significant progress has been made in ensuring safe therapeutic products derived from animal cells in bioreactors. However, the challenges posed by mobile genetic elements and among them ancient viral sequences continue to demand our attention.

This is a multi-part reminiscence on the emergence of an idea/concept. It is the result of many contributions by scientists over decades. The here provided text and images were executed with the help of Maria Wurm, Divor Kiseljak, Paco Pino, Concetta Cardone, Sergio Da Costa, Stéphanie Anchisi, Sebastian Rheindorf-Zaorski, Diogo de Jesus and Florian M. Wurm.
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**References**


1. Maertens, G.N. et al. (2022) Structure and function of retroviral integrase. Nature Reviews Microbiology 20, 20-34.
2. https://en.wikipedia.org/wiki/Alteplase
3. Ono, M. et al (1985) Nucleotide sequences of the Syrian Hamster Intracisternal A-Particle gene: Close evolutionary relationships of Type A particle gene to Type B and D oncovirus genes. J. Virol. 387-394.
4. Wurm, F.M. et al. (1989) Presence and transcription of endogenous retrovirus-like DNA sequences in CHO cells. In: Advances in Animal Cell Biology and Technology for Bioprocesses. Eds. R.E. Spier, J.B. Griffiths, J. Stephenne and P.J. Crooy, 76-81, Butterworths.
5. Anderson, K.P. et al. (1990) Presence and transcription of intracisternal A particle related sequences in CHO cells. J. Virol. 64 (5), 2021-2032.
6. Venter, J.C. et al. (2001). The sequence of the human genome. Science. 291 (5507): 1304–1351.
7. Duroy, P-O. et al. (2019) Characterization and mutagenesis of Chinese hamster ovary cells endogenous retroviruses to inactivate particle release. Biotech Bioeng. DOI: 10.1002/bit 27200
8. Li, S. et al. (2019) Proteomic annotation of the Chinese hamster reveals extensive novel translation events and endogenous retroviral elements. Journal of Proteome Research, 18(6), 2433–2455. https://doi.org/10.1101/468181
9. Naville, M., Volff, J.-N. (2016) Endogenous retroviruses in fish genomes: From relics of past infection to evolutionary innovations? Frontiers in Microbiology doi:3389/fmicb.2016.01197
10. Löwer, R. et al. (1996) The viruses in all of us: Characteristics and biological significance of human endogenous retrovirus sequences. PNAS 93, 5177-5184
11. Patel, M.R. et al (2011) Paleovirology – ghosts and gifts of viruses past. Curr. Opin.Virol. 1, 304-309
12. Reid, G.G. et al. (2002): Comparison of electron microscopic techniques for enumeration of endogenous retrovirus in mouse and Chinese hamster cell lines used for production of biologics. J. Virol. Meth. 108, 91-96
13. Stocking, C., Kozak, C. (2008) Murine endogenous retroviruses. Cell.Mol. Life Sci. 65, 3383-3398
14. Wurm, F.M. (2013) CHO Quasispecies – Implication for Manufacturing Processes. Processes 1,3, 296-311
15. Wurm, F.M., Wurm, M.J. (2017): Cloning of CHO Cells, Productivity and Genetic Stability – a Discussion. Processes 2017, 5, 20, doi: 103390/pr5020020