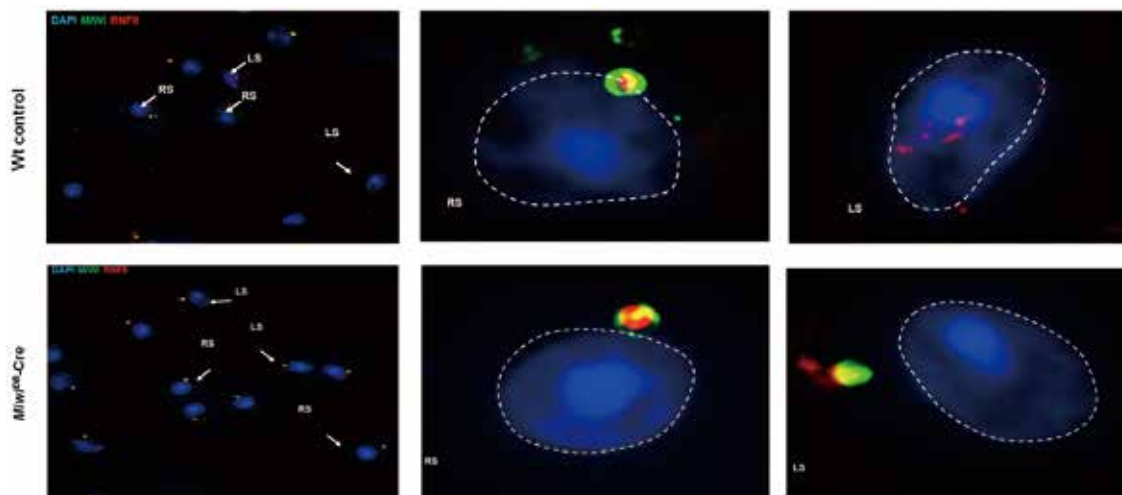


# Lost in Redundancy: Dysfunctional Sperm Found Victim of Failed Histone Replacement Caused by Mutant Human *Piwi*

By SONG Jianlan (Staff Reporter)

Less is more. It was never known that failure in ubiquitination, a process where proteins are tagged for later degradation, could cause disastrous loss in the end. In this case, the victim is the sperm, as well as its vigor.



A lab at SIBCB reports in top journal *Cell* that mutations at a “destructive” domain of human *Piwi* gene might be responsible for azoospermia — no sperm in semen. The images indicate that the defective proteins expressed by the mutant gene can impede the ubiquitination of histone and lead to severely decreased amount and activity of sperm. (Image by courtesy of LIU Mofang’s lab at SIBCB.)

**M**ale infertility, an issue that has aroused global concern in an era marked with ageing population and low birthrate, to a large extent results from decreased quantity and worsened quality of sperm, according to experts. An extreme case is idiopathic azoospermia, a medical condition featuring no sperm in semen. This remains an obstinate disease that has perplexed scientists, as its underlying mechanisms are far from clear and hence no well-targeted treatment strategy is available, let alone any effective therapy or medicine.

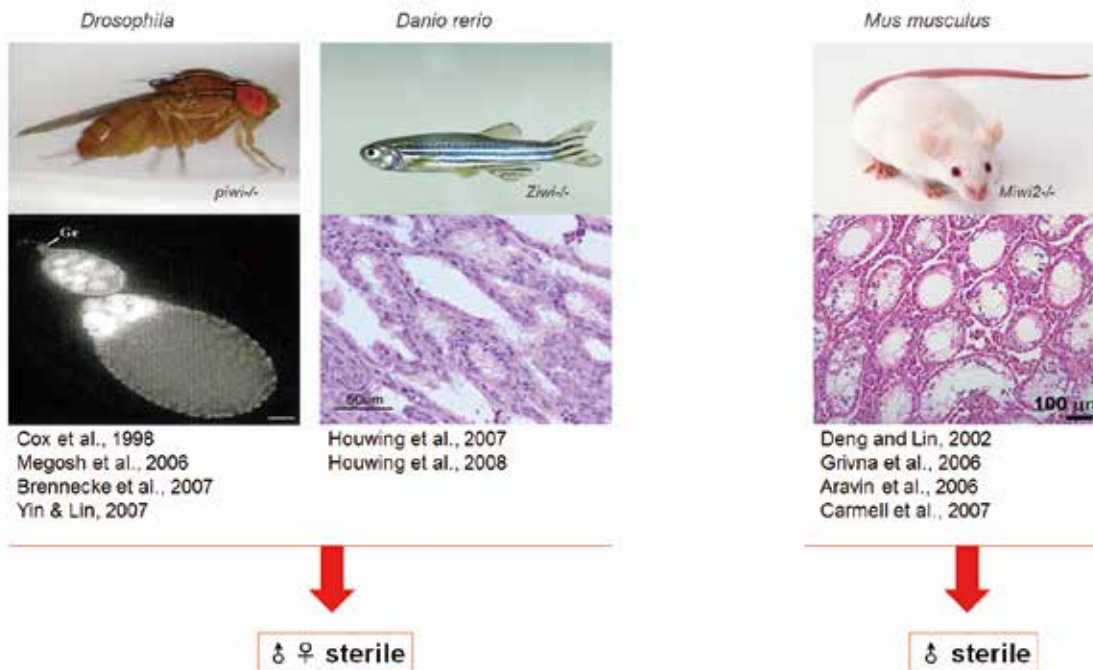
Thankfully, a recent study has locked onto the culprit of the disease, and accordingly proposed has been an effective treatment strategy, as a result from a seven-year joint venture of two women scientists in Shanghai, China.

In cooperation with Dr. SHI Huijuan at the NPFPC-Shanghai Institute of Planned Parenthood Research, Fudan University Reproduction and Development Institution, Dr. LIU Mofang at the Institute of Biochemistry and Cell Biology (SIBCB) under the Chinese Academy of Sciences (CAS) just reported the results from their long-term studies in *Cell* on June

1: mutations in a human gene called *Piwi* might be responsible for azoospermia. They accurately identified the mutations in a domain of the gene *Piwi*, and further revealed a cascade of sophisticatedly programmed regulatory episodes at the formative stage of sperm that eventually contribute to failure of male germ cell development and sperm generation.

What the team has unveiled brings in spotlight a low-profile regulation in germline development coded by a certain domain of *Piwi*, the ubiquitination – a drama meant to tag targeted proteins for degradation played by a peptide called ubiquitin. It would be hard to image what aftermath the failure in such a “subtraction” can invite if occurring in the developmental phases of male germ cells, had not for LIU and SHI’s work.

Unlike ubiquitination, another leading role in this drama enjoys much more recognition and attention. The gene *Piwi*, as introduced by Dr. LIU, has long been known for its pivotal role in the development of male germ cells. Nevertheless, it remained unclear whether or not it causes male infertility – until LIU and her cooperators’ discovery.



*Piwi* genes have been known to regulate germline development in animals, invertebrate and vertebrate. However, it remains unknown whether or not it is the disease gene for male infertility. (Image by courtesy of LIU Mofang’s lab at SIBCB.)

## Dubious *Piwi*

As an evolutionally conserved family of biofunctional molecules, *Piwi* proteins can be found in the germline of many invertebrate and vertebrate animals, from worms, flies, fishes, to mice and humans, and earlier genetic studies had made clear that the family members are of critical importance in the formation and maintenance of germline stem cells. Particularly, they are highly expressed in the testes of mice, and are accordingly required for male but not female fertility; similarly, they are also mainly expressed in the testes of humans, though their specific roles in human male germ cell development are still not clear.

It is known that *Piwi* proteins contain four domains, and the function of three out of the four domains has been elucidated in earlier research. However, the function of the fourth, *i.e.*, the N-terminal domain, remains elusive. Could this hermit actually be the long-sought-after culprit?

In their work, LIU's team identified a conserved destruction box (labeled as "D-box") in the mysterious N-terminal domain of vertebrate *Piwi* proteins, including that in mice (MIWI) and humans (HIWI). Based on the discovery that the ubiquitination and degradation of MIWI are dependent on D-box, plus the important role of ubiquitination and degradation of MIWI in male germ cell development, the team suspected that this mysterious domain might be liable for mutations contributing to male infertility.

The team hence embarked on a screening for potential mutations in this special domain in human *Piwi* gene (dubbed "*Hiwi*") in a total of 413 patients of idiopathic azoospermia. What they found confirmed their suspicion: they identified three different D-box mutations in three patients, all in this tricky domain.

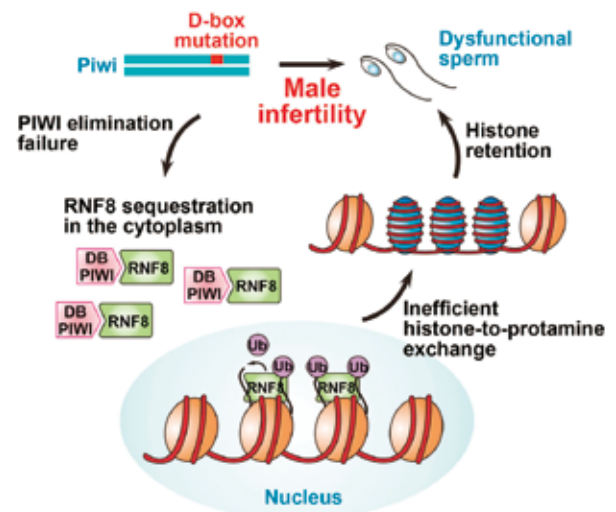
To make clear whether these mutations are the cause of azoospermia, the researchers knocked the identified D-box mutations in mouse *Piwi* (*Miwi*) and examined what happened in the mouse model. They observed that all the mice carrying the mutant D-box demonstrated male infertility, manifesting the same phenotype as the human patients.

Further, via modeling such mutations in both knockin and transgenic mice, the team found that such mutations in D-box can impeded the development of spermatids, resulting in deformed sperm with aberrant shape, porous structure, and severely impaired activity,



*Dev Cell*, 18:371–384, 2010

RNF8 plays a key role in histone-to-protamine exchange. (Image by courtesy of LIU Mofang's lab at SIBCB.)



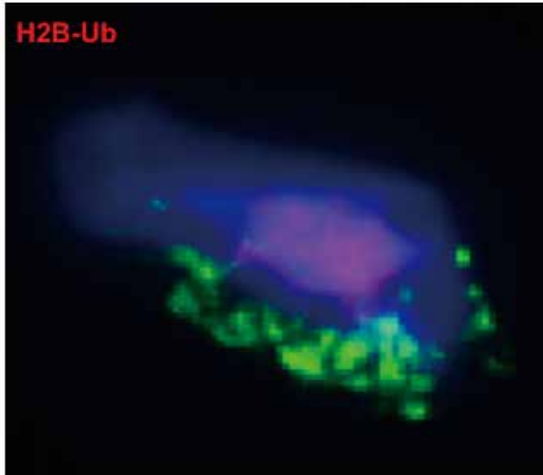
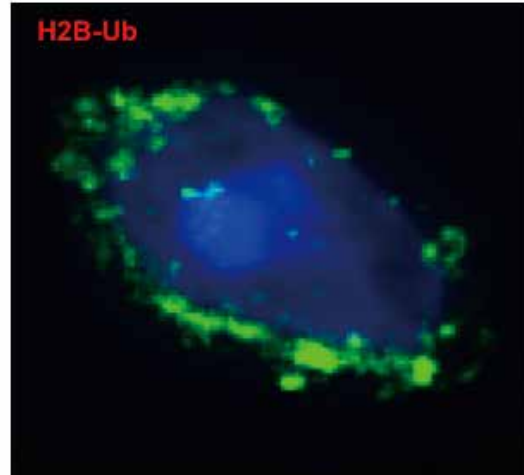
A step-wise illustration showing how the defective proteins expressed by the mutant D-box (*Piwi*) lead to the production of dysfunctional sperm. (Image by courtesy of LIU Mofang's lab at SIBCB.)

though still a small amount of sperm can be generated.

It is hence verified that the mutations in D-box of *Piwi* are the causal genetic defect of male infertility. But how come these defects can lead to debilitated sperm, or even no sperm in semen? What could this "destructive" box do with sperm?

## Less Is More

Interestingly, the defective MIWI, as what the stray D-box mistakenly codes, can prevent some proteins –

**RNF8-N:GFP****RNF8-N<sup>mut</sup>:GFP**

RNF8-N, a fragment of RNF8, comes for rescue. Ubiquitination of histone is observed restored in the experiment after transduction of RNF8-N. (Image by courtesy of LIU Mofang's lab at SIBCB.)

which are otherwise supposed to be removed from the sperm – from being “destroyed”.

Among them is histone, which works as a frame or “lattice” in the nucleus, containing and protecting DNAs. In typical somatic cells, the genomic DNAs are organized on histone octamers in the nuclei. The resulted array of DNAs, however, would be too big for the sperm in many animals to accommodate.

Frequently likened to a tadpole for its special shape, sperm features a tiny, streamlined head and a linear, thin tail. This peculiar structure guarantees optimal hydrodynamics in semen, which is vital for its triumph in the dashing swimming race to the egg – after all, only the first prize winner(s) of the competition can grasp the extremely slim chance to fertilize the egg.

The tiny volume of the head makes it necessary to condense the whole genome within a very limited space; hence more compact a DNA “frame” is needed. This is where protamine makes a difference: this type of small and highly alkaline proteins is able to fold and package DNAs into a much smaller volume fitting in the head.

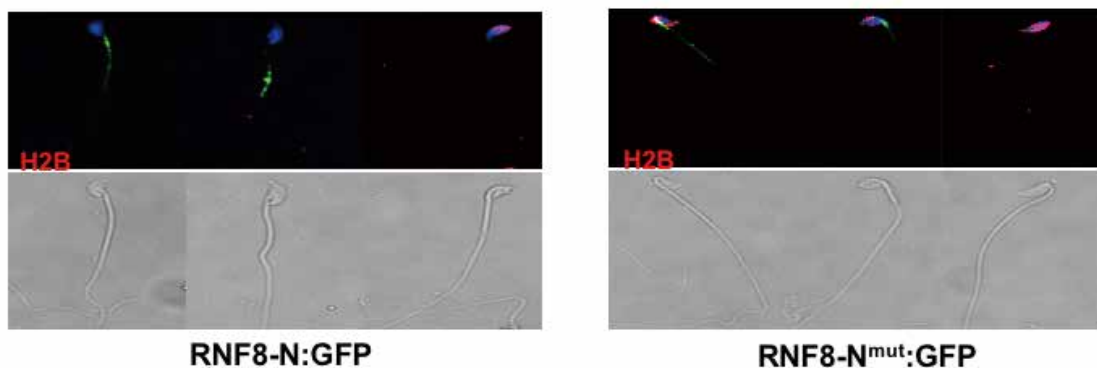
What follows is a very different ingredient composition of the nuclei – more protamine and less histone; therefore, the majority of histone must be replaced by protamine during the formation of sperm.

### Dysfunctional Sperm

The removal of histone relies on a kind of ubiquitin ligase that specifically targets at histone in nucleus, namely the RNF8. At a later phase of sperm formation, this enzyme catalyzes the mono-ubiquitination of histone to facilitate its replacement by protamine in sperm head. This indispensable enzyme, unfortunately, is absent from the nucleus at this vital stage of sperm development in mice carrying the mutant D-box, as observed by LIU's team in knockin mice model.

Due to the defect in mutant D-box, MIWI fails to decompose in the late developmental stages of spermatids; moreover, it sequesters RNF8 in a complex, forcing it to retain in the cytoplasm, and hence fails to enter the nucleus to feed histone with necessary signal to be replaced by protamine. Following this deprivation, the mono-ubiquitination of histone fails: replacement of histone is insufficient, and the retentive/residual histone in the nuclei clogs the pathway for histone-protamine exchange.

As a result, absence of RNF8 in the nucleus directly impeded the histone-protamine exchange. The failure not only increases the size of the sperm head and deprives the sperm of good hydrodynamics, but also increases the chances of DNA damage. Given that successful histone-protamine exchange can erase



Transduction of RNF8-N, a fragment of RNF8, is found boosting the removal of histone in sperm heads. The sperm subsequently produced are also found rejuvenated. (Image by courtesy of LIU Mofang's lab at SIBCB.)

some epigenetic markers associated with histone modifications, its failure could also lead to anomaly in other regulations.

All the failure in subtraction leads to more losses instead of adding any benefits. In the end, mutations in D-box, the “destructive” box of *Piwi*, largely reduce the amount of sperm, and produce very little sperm of poor quality – all in abnormal shape, and severely compromised activity.

### Potential Therapy

In humans, as introduced by Dr. LIU, azoospermia affects about 1% of the male population, and up to 20% of male infertility situations could be attributed to this disease. In China, non-obstructive azoospermia, asthenospermia and teratozoospermia are important causes of male infertility, according to incomplete statistics. Naturally, filling the void for effective treatment for these diseases has been a long-pursued goal for scientists.

Now LIU's team made it. They offered a potential therapy: they found a truncated peptide that is able to rescue RNF8 from its unlucky tie with MIWI.

In their further research, the team found that a peptide fragment from RNF8 itself, named RNF8-N, can compete with the full length RNF8 to bind with MIWI, hence freeing it for later catalysis in histone-protamine exchange. Therefore, potentially this truncated RNF8 can work as a cure for the disease.

It took the team seven years to arrive at the results, but it is worth it. “It is rarely seen in literature that a research gives such a neat model for application in translational medicine,” commented Dr. WU Ligang, LIU's colleague at SIBCB. “It results from long-term accumulation of experience and knowhow from multiple disciplines,” concluded CAS Member Prof. WANG Enduo, well-known expert in interaction between enzyme and nucleic acids at SIBCB. She thought highly of the contribution of LIU and collaborators to human reproductive health, a far-reaching issue of today.

#### For more information please refer to:

Gou LT, Kang JY, Dai P, Wang X, Li F, Zhao S, Zhang M, Hua MM, Lu Y, Zhu Y, Li Z, Chen H, Wu LG, Li D, Fu XD, Li J, Shi HJ\*, Liu MF\*. Ubiquitination-deficient Mutations in Human Piwi Cause Male Infertility by Impairing Histone-to-Protamine Exchange during Spermiogenesis. *Cell*, 2017;169:1090-1104.