Biological dual-use research in synthetic genomes

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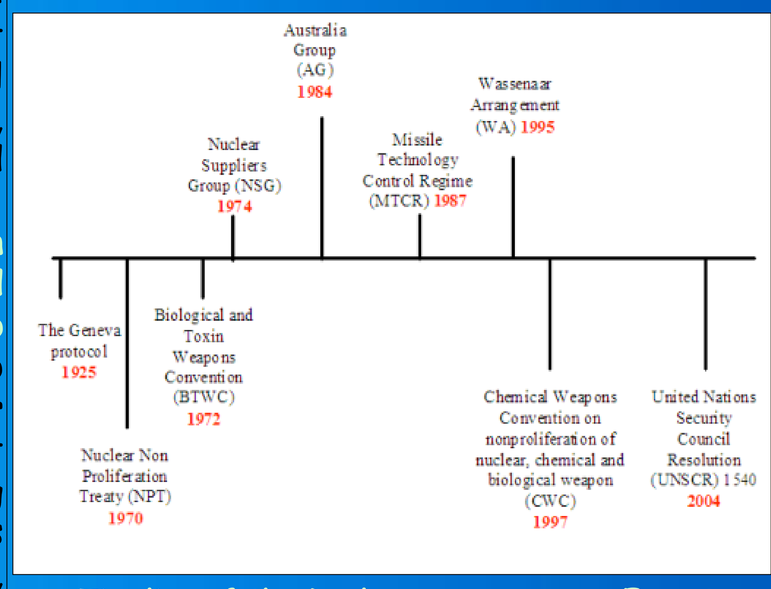
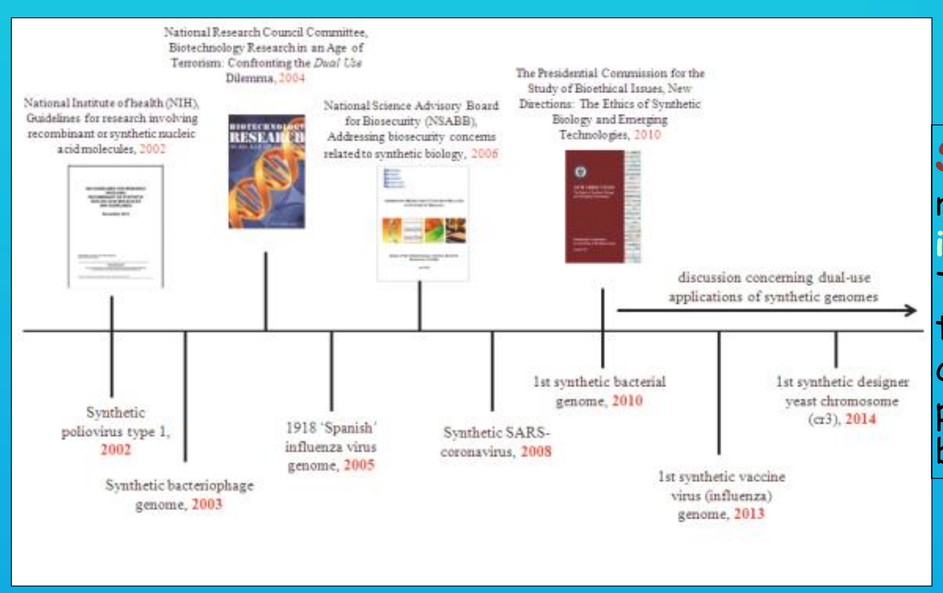
In recent years, the publication of the studies on transmissibility in mammals of H5N1 influenza virus and synthetic genomes has triggered heated and concerned debate on **biological dual-use research** within the scientific community; these papers have raised the awareness **that in some cases fundamental research could be diverted to harmful experiments with bioterrorism purposes.** The term dual-use has multiple meanings. Recently, the term started to be used in non-proliferation legislation, like export control laws, **to address the problem that technologies or knowledge might be used for proliferation purposes; this includes materials, hardware, intangible technology that have peaceful application but could also be exploited for illicit production of biological, nuclear or chemical weapons (CBRN).**

International agreements on dual-use control. In order to counter the production, and eventually the use, of Weapons of Mass Destruction (WMD), international agreements were signed. The most important treaties are the Geneva protocol (1925), the Nuclear Non Proliferation Treaty (1970), Biological and Toxin Weapons Convention (1972), the Chemical Weapons Convention on nonproliferation of nuclear, chemical and biological weapons (1997). The concept of Dual-use was defined in the aforementioned fora and developed in the Nonproliferation Export Control Regimes.

Despite the fact that Regimes are less known to public opinion than treaties, they are very active in the prevention of production of WMD by controlling the exports of those dual-use products and technologies that might have an unacceptable risk of diversion. Here we focus on the **Australia Group (AG) Regime** because it controls the exports of chemical and biological materials. The Australia Group Regime was set up to address the concerns raised by the use of chemical weapons during the Iran-Iraq War (1981-1988). The goal of the AG is to develop ways to minimize export and transhipping risks so that proliferators would not be able to obtain the necessary inputs for chemical and biological weapons. Enforcing licensing authority over a wide range of chemical weapons precursors is one way to reduce risk. Members States (42 as of November 2015) require licenses to export dual-use chemical manufacturing facilities, equipment, and related technology, plant pathogens, animal pathogens, biological agents and dual-use biological equipment.



Timeline of the Dual-use governance. Export control Regimes are shown above the timeline whereas Treaties and UNSCR are reported below the timeline.

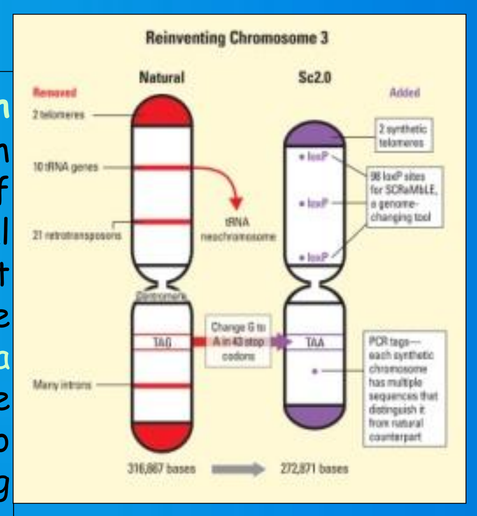


Timeline of synthetic genomics and principal guidelines on biosecurity

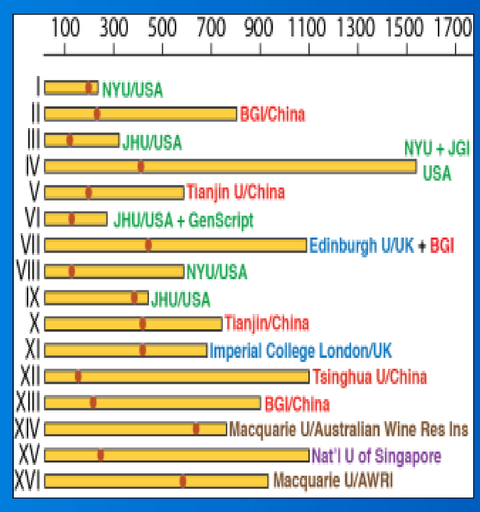
Synthetic genomes. New evolving biological technologies, such as synthetic biology, receive great attention; **synthetic biology is widely used to create new metabolic pathways in bacteria (Escherichia coli) or unicellular eukaryotes.**

The interest in synthetic biology research as a proliferation risk made a leap forward when the first synthetic bacterial chromosome and the first synthetic living organism were created. The possibility to create a synthetic genome from scratch could inspire the production of a human pathogenic virus (the first synthetic virus was created in 2002) or a bacterium for which no vaccine is available.

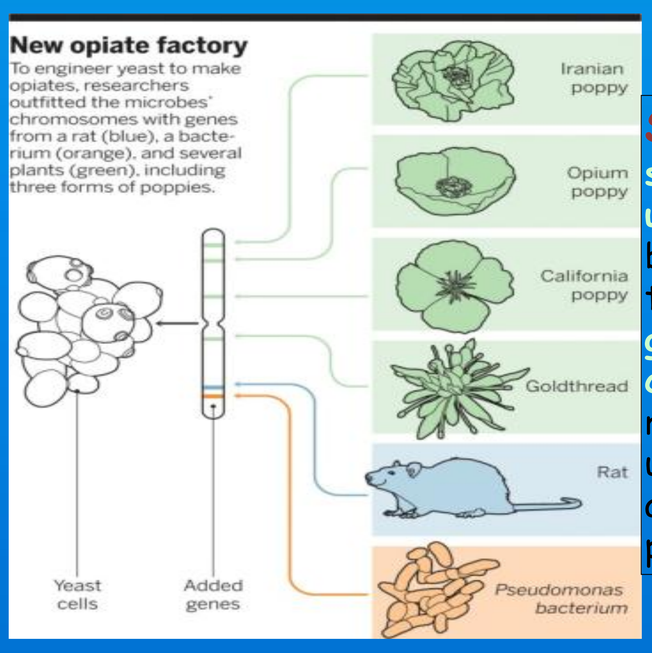
The first eukaryotic synthetic genome. The interest in **synthetic biology research as a proliferation risk** made a leap forward when the first synthetic synthetic living organism were created. In 2014, the first synthetic yeast chromosome was produced this result is part of a collaborative project called Sc 2.0, engaging scientists from academic and commercial institutions across the globe to produce the first eukaryotic synthetic genome. The yeast synthetic chromosomes will have unique features, allowing to reshuffle and/or eliminate nonessential genes from the genome, making possible genome reduction and the construction of a minimal cell factory for industrial applications. The value of this technique is to create engineering cells for efficient production of a desired compound; this aim often fails due to inhibitory interactions caused by the introduction of the heterologous pathway into an existing natural metabolic network.



The successful reinvention of yeast chromosome 3 involved the removal of many elements and multiple additions to its DNA



State of the art of the Sc 2.0 project: six chromosomes were completed at Sept 2015



Synthetic biology. New evolving biological technologies, such as synthetic biology, receive great attention; **synthetic biology is widely used to create new metabolic pathways in bacteria (Escherichia coli) or unicellular eukaryotes, such as the yeast *Saccharomyces cerevisiae*,** to produce new compounds such as biofuel or antibiotics. Just to mention few examples, yeast was recently used as biomanufacturing platform for the synthesis of the **antimalarial artemisinin precursor; in 2015, different groups successfully inserted genes from E.coli, opium poppy sugar beet and mouse in yeast, reconstructing the pathway for morphine or codeine production.** The possibility to reconstruct the entire pathway in a single yeast cell has now become a reality and has opened the **possibility to misuse this achievement;** the researchers, aware of the possible dual-use of these strains, spontaneously contacted experts in biotechnology policy enlightening the possibility that anyone with access to morphine producing yeast strain and with basic skill for fermentation, would be able to produce morphine easily starting from glucose.

In the short to medium term, synthetic biology is unlikely to pose new risks or threats, but it could lead to consider a risk also harmless organism such as Saccharomyces cerevisiae with a **long-term possibility to insert synthetic genes, not present in the natural organism, with the aim to produce biological weapons or drugs.** In the future, the advanced biotechnological capabilities might become more accessible to non-experts and indeed the Sc 2.0 project is accomplished by an international team made up mostly of undergraduate students. In a near future, it will be possible to determine the **minimal genome necessary for life using it as a versatile platform to express easily synthetic pathways,** bypassing the physiological cellular limit to produce new compounds: **not only antibiotics, pharmaceutical agents or biofuel, but also toxic agents or drugs.**

Since the researchers exchange biological materials (i.e. plasmids, strains, antibodies, nucleic acids) and intangible technology, they should be sensitised on this topic to draw the attention on the so called Dual-Use Research of Concern (DURC) as well as the principles and activities of the Australia Group (AG), which focuses on chemical and biological dual-use materials export control.

Ref. A. Cirigliano, O. Cenciarelli, A. Malizia, C. Bellecci, P. Gaudio, M. Lioj and T. Rinaldi. Dual use concept and dual use research in synthetic genomes, submitted for publication to Science and Engineering ethics.