Computational Systems Biology
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Lecture - 68
Perturbations to Metabolic Networks: Synthetic Lethals

In today's video, we will look at a very interesting concept called synthetic lethals. Synthetic lethals are sets of genes or reactions where you need to remove all of them together to abolish growth in a cell. So, if you remove a single gene nothing happens, but if you remove a pair of genes the organism dies. So, how do you identify these combinations from a metabolic network. We will go into greater details in a moment.

We have been looking at perturbations to metabolic networks. Let us look at an important type of perturbation, we have looked at deletions and over-expressions. Let us look at another important type of perturbation, which is multiple deletions and which leads us to the concept of what is known as a synthetic lethal.

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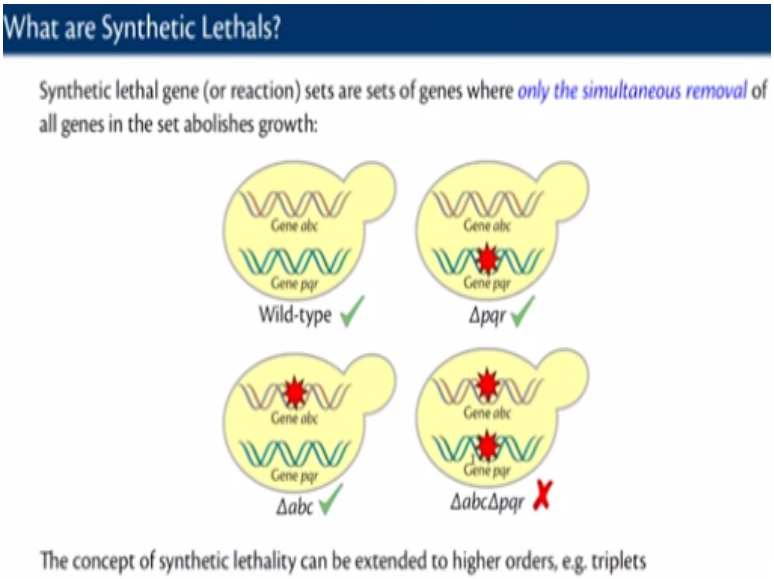

What are synthetic lethals? They are sets of reactions or genes when only the simultaneous removal of all the genes in a set abolishes growth of the organism. Let us consider the simple example here. There are two genes abc and pqr. When you delete abc as you see in the bottom, nothing happens. When you delete pqr alone, organism still grows. When you delete both genes

together, the organism dies. So, you synthetically superimpose the two deletions essentially. You can also extend the concept to higher orders.

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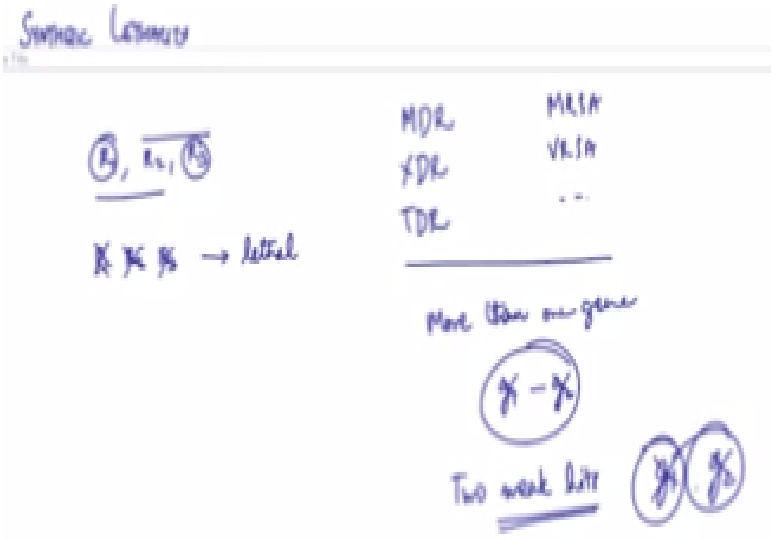

Let us say there are reactions R1, R2, R3. When I remove these two nothing happens. When I remove these two nothing happens. When you remove R1 and R3, the organism continues to survive. When you remove R1, R2, R3, it is lethal. You can also therefore extend it to quadruplets as well. If you remove 3 at a time, 2 at a time, 1 at a time, nothing happens. When you remove 4 at a time, the organism no longer survives. This concept is known as synthetic lethality.

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Why do we need to identify synthetic lethals? What are the use cases? First thing it help us understand gene function and functional associations. It is kind of easy to find out what genes are isozymes for one another and so on. But, through this analysis you can find two reactions that are in different subsystems that could potentially be compensating for one another. It is possible to find genes or reactions that are sort of far apart, which can compensate for one another.

Synthetic lethals are also useful to identify combinatorial drug targets and pathogens. This is again a matter of intense research and debate. It is common to have organisms; especially bacterial organisms develop resistance. Especially in diseases such as tuberculosis, you must be familiar with many of these acronyms thus MDR, XDR, TDR. Multidrug resistant tuberculosis, extensively drug resistant tuberculosis, totally drug resistant tuberculosis, resistant to all known drugs.

Similarly, you have MRSA, VRSA and so on. Methicillin-resistant Staph aureus, vancomycin resistant Staph aureus and so on. The idea is could you in any of these cases can you target more than one gene. This makes it that much more difficult for the organism to develop resistance, but there still remains a question, should you target a synthetic lethal or two single lethal genes. You remove g1, the organism dies. You remove g2, the organism dies. You could also look at this as a pair to target.

But the problem is there is a lot of benefit to adapt to this mutation. In other words, whenever you throw a drug at an organism, you are always selecting and there is a strong selection pressure to kill the organisms that are weaker. It is like you just take your mosquito spray and you spray it, you are trying to kill mosquitoes at the same time you are selecting for resistant mosquitoes. When you put two sprays you will find mosquitoes are resistance to both sprays and so on.

Here the idea is the flip side of it is that, if you have two weak hits, g1. If you remove g1, nothing happens. So, there is no major selection pressure arising out of g1. If you remove g2, nothing happens. No major selection pressure. You remove g1 and g2, the organism dies. These are two weak hits that can essentially trick the organisms, so there is some interesting studies about how weak hits can confuse complex systems.

The idea is you give 2 hits that are not very strong. You are not targeting essential genes, but you are targeting important genes, which the organism does not truly require for survival except together. An even better case for the application of synthetic lethals is in cancer therapy. Cancer invariably involves some gene expression gone awry, some genes gone awry. What you find is you may have a cancer cell where in it is already missing a particular gene.

The loss of that potential tumor suppressor gene has induced the development of cancer in this cell. So, synthetic lethal is not singly lethal.

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So the thing is let us say this is a health cell and this is a cancer cell. The cancer cell already lags g1. The healthy cell has g1. If you target g2, which is synthetically lethal with this in both, the healthy cell will continue to live, the cancer cell will die. Because the cancer cell has already lost g1 in the process of developing cancer, may be that is the reason it developed cancer or whatever.

This is a very interesting aspect of synthetic lethals. Not that you can use metabolic networks to find synthetic lethals in cancer, but I think that should be possible once you have better curetted models and potentially better objective functions to study larger organisms. Because the objective function challenge still very much remains. How do you actually find the most correct objective function for a complex cell?

For a bacterial cell it is easy, maximize growth, cell is happy, we are happy because we get good predictability. But, what about more complex organisms. Cancer therapy is another possible application for synthetic lethals. How do you identify synthetic lethals? What would be the simplest way to identify synthetic lethals? Let us first look at experimental methods before we think about computational methods.

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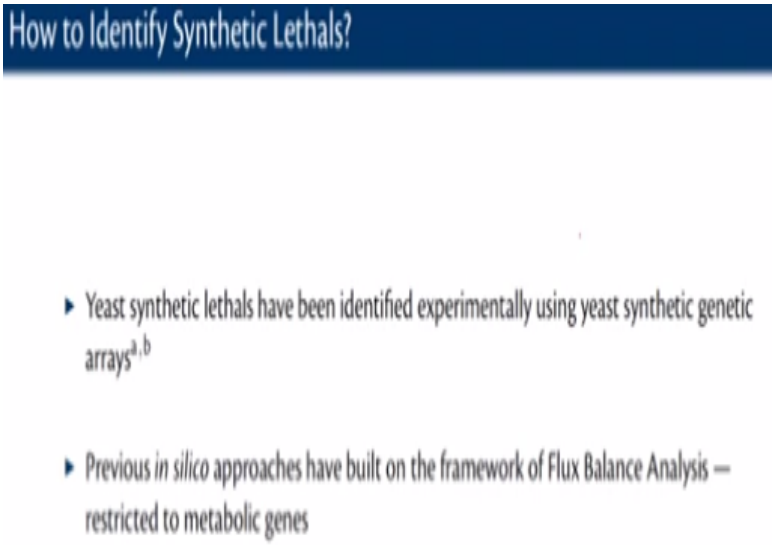

E synthetic lethals have been identified using E synthetic genetic arrays, so large high through put phenotypic screens. We just remove multiple genes at a time and check the phenotype. Previous in silico approaches also have built on the framework of FBA, but of course it is restricted to metabolic genes always.

All through the last few classes we have been restricting ourselves to metabolic genes. You cannot say what happens when you remove a non-metabolic gene. How would you identify synthetic lethals? What would be the naive way to identify synthetic lethals? **"Professor - student conversation starts"** What (()) (09:06) removing one gene. You just remove all pairs of genes.

You have n genes in an organism, you make n choose to deletions and you can run an FBA for each of those cases or even a MOMA or ROOM and then figure out whether you are getting a growth or not. **"Professor - student conversation ends"**

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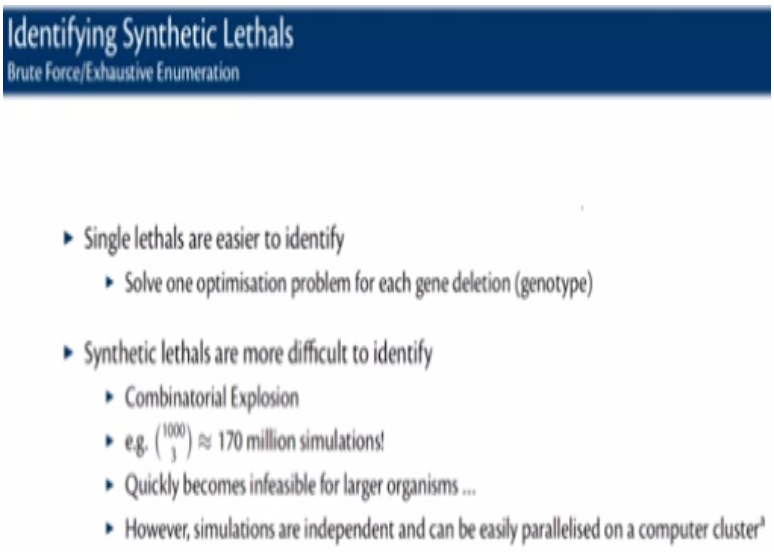

Single lethals are actually even easier to identify, it is just solving one optimization per deletion. Synthetic lethal there is a combinatorial explosion. If you have 1000 genes you are talking about 1000 choose 2, which is half a million. The problem is 1000 choose 3, which could be as high as one-fifth of a billion. So, this quickly becomes infeasible for larger organisms, but given the simulations are independent, it is easy to parallelize these across the cluster. This is commonly done.

You just offload all this heavy computation to multiple cores, which can do the job in parallel. Because none of these simulations are dependent on one another. It is about setting V1V2 equal to 0 in one simulation, V1V3 equals 0 in another, V1V4 equals 0 in another simulation. So, these are the constraints that you are throwing and literally the same core linear programming problem. The core LP is the same. Maximize transpose V such that SV equal to 0 subject to varying additional constraints on what is being deleted and so on.

"Professor - student conversation starts" But C will be same right. C is the same. **"Professor - student conversation ends"**

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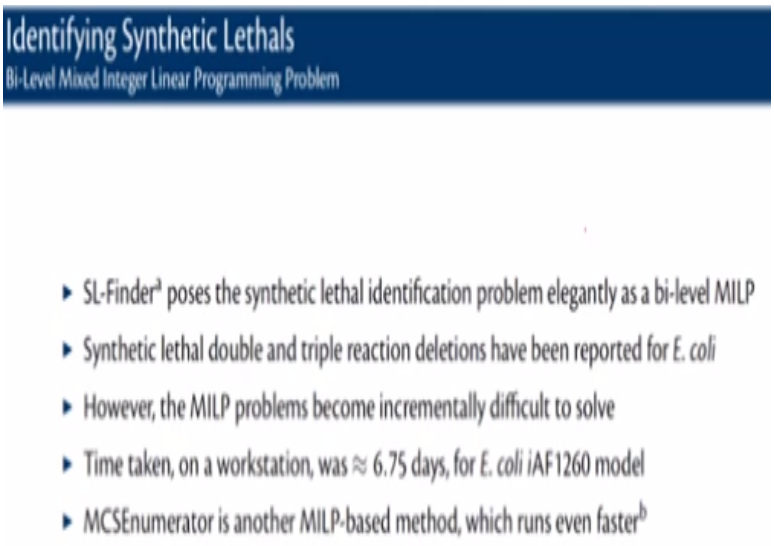

So there are previous approaches that have used bi-level mixed integer linear programming problems. There is this approach called SL-Finder, which poses the synthetic lethal identification problem very elegantly as a bi-level mixed integer linear programming problem, so they identify synthetic lethal double and triple reaction deletions in E. coli. But, the problem with MILP is that they become incrementally difficult to solve.

How this works is I will not get into the formulation details, you can look it up. It first solves, gets one synthetic lethal. It tries to solve to solve an integer problem by saying delete at most two genes to give me a lethal phenotype. But, while doing that to get the next lethal you have to do what shows an integer cut. Something the equivalent of saying give me something other than the first solution. The next time you have to tell me give me something other than the first or the second solution and so on.

So, the problem becomes incrementally difficult to solve and they took about one week to solve this on a workstation. But, then a newer approach was developed called MCS Enumerator. This is another MILP based method, which runs much faster. But the issue with this in general is that you cannot parallelize these. Because you want to use one solution, then say do not give me that solution and give me a better solution kind of thing.

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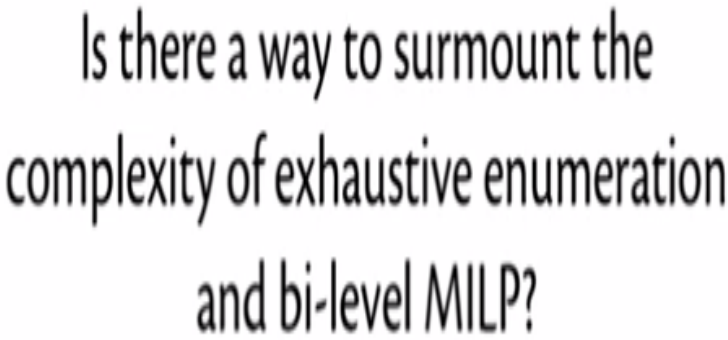

The question we wanted to ask was, is there a way to surmount the complexity of exhaustive enumeration and bi-level MILP? Exhaustive enumeration is obviously very difficult to do 170 million simulations if you are trying for triple lethals. Similarly, it is pretty difficult to do bi-level MILP problem solving as well. So, is there a way to get around this.

I hope you got a good overview of the concept of synthetic lethals today and how different approaches have been used to identify synthetic lethals. I also must have convinced you that these approaches are a little computationally expensive and we need alternative approaches. So, in the next video, I will talk to you about a very interesting algorithm that was developed in our lab for computing synthetic lethals namely the Fast-SL algorithm.