Navigating the Shallows and Rapids of Cholesterol Synthesis Downstream of HMGCR

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Summary  
Cholesterol is vital for human life, but its levels must be tightly regulated. Too little cholesterol leads to developmental disorders, but too much is widely appreciated as contributing to heart disease. Levels are regulated through the coordinated control of cholesterol synthesis, uptake and efflux. Here, we focus on cholesterol synthesis. The cholesterol synthesis pathway involves more than twenty enzymes, but most research so far has focused on a very early enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), a well characterised control point. This is largely because HMGCR is the target of the successful cholesterol-lowering drugs, the statins. Our recent work has examined several other enzymes in the pathway and revealed complex regulatory mechanisms that also contribute to the control of cholesterol synthesis. In this review, we discuss the transcriptional regulation of the two terminal enzymes, 7- and 24-dehydrocholesterol reductase (DHCR7 and DHCR24), where we have found that a cooperative transcriptional program exists. We also discuss the post-translational regulation of another critical enzyme, squalene monooxygenase (SM), which has its protein levels controlled by cholesterol, and DHCR24, which has its activity affected by sterols and related compounds, as well as via phosphorylation/signalling. There is an unforeseen complexity in the regulation of cholesterol synthesis which requires further investigation.

Key Words  
cholesterol synthesis, DHCR24, DHCR7, squalene monooxygenase, regulation

The general perception of cholesterol is negative, largely owing to its status as a classical risk factor for heart disease. But the flipside is that cholesterol is vital for humans, being an essential component of membranes (including specialised domains), as well as providing the starting material for bile acid and steroid hormone production. The duplicitous nature of cholesterol means that we have evolved elaborate mechanisms to keep this lipid in careful balance. This is achieved at the cellular level by balancing cholesterol export with cholesterol uptake and synthesis. In recent years, our laboratory has been dedicated to understanding how the cholesterol synthesis pathway is regulated beyond the best known control point, HMGCR [reviewed in (1)].

DHCR7 and DHCR24: Evidence of a Cooperative Transcriptional Program in Cholesterol Synthesis

At the transcriptional level, cholesterol homeostasis is largely mediated by the SREBP-2 transcription factor. The binding sites for SREBP-2, known as sterol regulatory elements (SREs), are typically found in the proximal promoter of the gene, within 1 kb of the start site (5). Since SREBP-2 is generally a poor activator of transcription by itself, it is typically found in close proximity to cofactor sites such as nuclear factor-Y (NF-Y) to help up-regulate gene expression (6).

DHCR7 and DHCR24, the terminal enzymes in cholesterol synthesis via the Kandutsch-Russell and Bloch
Beyond HMGCR

pathways respectively, are both under the control of SREBP-2. Unlike most target genes which possess only one SRE site, dual SREs have been mapped in the human promoters of several cholesterol biosynthesis genes, including DHCR7 (7) and DHCR24 (8). These dual SRE sites were identified as working cooperatively to activate gene expression, suggesting SREBP-2 levels must reach a critical threshold before transcription is initiated. Considering that cholesterol is energetically expensive to produce, this ensures that resources for cholesterogenesis are only available when there is high demand for cellular cholesterol. Thus, this unique cooperative system helps to control the transcriptional expression of these genes and regulate total cholesterol levels.

SM: A Post-Translational Control Point Post-HMGCR

Squalene monooxygenase (SM) catalyses the conversion of squalene to 2,3-oxidosqualene. This step lies immediately after the first committed step of cholesterol synthesis, the formation of squalene by squalene synthase, and precedes the cyclisation step that forms the first sterol intermediate, lanosterol (9).

In addition to transcriptional regulation by SREBP-2, SM is post-translationally regulated. Like HMGCR, SM is a rate-limiting enzyme in cholesterol synthesis (10, 11). However, SM lies downstream of HMGCR, and importantly, beyond the isoprenoid branch point. While HMGCR is post-translationally regulated by non-cholesterol sterols, including 24,25-dihydrolanosterol (12) and oxysterols (13), SM is directly regulated by cholesterol itself (10), which results in its degradation via the ubiquitin-proteasome system. MARCH6 has been identified as the E3 ligase responsible for this targeted degradation (14, 15). Cholesterol-dependent degradation of SM can be reversed by unsaturated fatty acids, which stabilise SM by preventing its polyubiquitination by MARCH6 (16). Post-translational down-regulation at the SM control point means that synthesis of cholesterol can be halted while allowing the formation of biologically important pre-squalene intermediates, notably the isoprenoids.

DHCR24: Transcriptional and Post-Translational Regulation

24-dehydrocholesterol reductase (DHCR24) catalyses the final step in cholesterol synthesis via the Bloch pathway, converting desmosterol into cholesterol. DHCR24 is involved in multiple cellular functions such as regulating oxidative stress, and is proposed to be neuroprotective, anti-apoptotic, and anti-inflammatory [reviewed in (17)]. Defective or deficient DHCR24 leads to the autosomal recessive disease, desmosterolosis, which results in multiple developmental anomalies (18). Thus, DHCR24 is a very important enzyme not only in cholesterol production, but also in other aspects of human health and disease.

As described, DHCR24 is transcriptionally regulated by sterols via the SREBP-2 transcription factor (8). It is also regulated by sex hormones and epigenetic factors at the transcriptional level. In addition, its regulation at the post-transcriptional level has been explored. Phytoestrogens (plant sterols), progesterone, and similar progestins were found to inhibit DHCR24 activity (19, 20). The oxysterol, 24(S),25-epoxycholesterol (24,25EC), produced in a shunt of the cholesterol biosynthesis pathway, also inhibits DHCR24 enzyme activity without affecting its protein expression (21). Recently, signalling via phosphorylation was implicated in the post-translational regulation of DHCR24 (22). Mutating DHCR24 phosphorylation sites (T110, Y299, and Y507) resulted in decreased DHCR24 activity. Furthermore, inhibiting a major kinase (protein kinase C, PKC) resulted in decreased DHCR24 activity. However, the site(s) for PKC and kinase(s) for the phosphorylation sites remain elusive. Post-translational regulation of DHCR24, which is at the distal end of the cholesterol synthesis pathway, provides a rapid means of turning cholesterol synthesis on or off.

Conclusion

The regulation of cholesterol synthesis enzymes involves a wide variety of mechanisms at both the transcriptional and post-translational levels. In this review, we have explained some of these mechanisms and how they relate to particular enzymes, notably HMGCR, SM, DHCR7 and DHCR24. However, there is still further research required to fully understand how these enzymes are regulated, and whether other cholesterol synthesis enzymes have similarly intricate modes of regulation.

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